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HOW ACCESS TO LANGUAGE AFFECTS CONTAMINATION SENSITIVITY: A STUDY OF FOOD REJECTION BEHAVIOUR IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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Abstract

To investigate the role of language in the emergence of contamination sensitivity, we studied food rejection behaviour in children with Autism Spectrum Disorders (ASD), who are known to be impoverished in language and communication. In Study 1, we demonstrated that contamination sensitivity is lacking in a subgroup of children with ASD but not in the majority of typically developing children (TD) or children with learning disabilities, who refused to drink juice that had been in contact with an insect. In Study 2, we established that the lack of contamination sensitivity found in a subgroup of children with ASD is linked to a deficit in auditory processing. In Study 3, using an eye-tracking paradigm, we explored to what extent the subgroup of children with ASD who lack contamination sensitivity in behavioral tasks are implicitly sensitive to contaminants. The results showed that TD children and children with ASD possessing contamination sensitivity had a looking preference for an uncontaminated drink in sharp contrast to children with ASD who did not possess contamination sensitivity. Moreover, TD children and children with ASD possessing contamination sensitivity showed a dilation in pupil size in response to a contaminant, while the subgroup of ASD children who lack contamination sensitivity didn’t show this reaction. Finally, in Study 4 we investigated the effect of language on implicit contamination sensitivity, by pairing explicit linguistic information about contaminants with the visual stimuli used in Study 3. The results showed that, while TD children and children with ASD with contamination sensitivity were sensitive to language, children with ASD who lack contamination sensitivity ignored such messages. However, pupil size increased also in the subgroup of children with ASD who lacked contamination sensitivity, even though of a less extent compared to the children of the other groups. As the two subgroups of ASD children in Study 3 and 4 did not differ in verbal and nonverbal mental age and Theory of Mind, but only in auditory processing (which was lower in ASD without contamination sensitivity), access to language seems to play a major role in contamination sensitivity.

Publications

Michael Siegal, Roberta Fadda, Paul G. Overton (2011). Contamination sensitivity and the development of disease-avoidant behaviour. *Philosophical Transactions of the Royal Society B – biological sciences,* 366, 3427-3432.

Posters and Presentations

- Roberta Fadda, Michael Siegal, Paul G. Overton (2010). Contamination sensitivity in children with autism. *Poster presented at the BPS Developmental Psychology Section Conference,* Goldsmiths, University of London, 12-15th September.

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THESIS OUTLINE

One of the most important themes in developmental psychology is the possible effects of linguistic experiences on the origins of biological concepts in children. Scientists have extensively investigated whether it is language that enables children to reason about causality and to develop an understanding of the causal mechanisms beyond superficial appearances in biology, chemistry and physics (Siegal and Surian, 2012). This kind of research has a rather relevant implication for children's well being, since biological knowledge forms the basis for the development of effective disease-avoidant behaviours. Together with the emerging cognitive abilities and affective reactions to contaminants, language and culture shape children's knowledge about disease. It is through language and conversation that children learn to link the perceptual cues of contamination to the actual threats of diseases. These links in turn operate as important determinants of disease-avoidant behaviours in daily life, triggered by affective reactions to contaminants which belong mainly to the realm of disgust (Siegal, Fadda, and Overton, 2011).

Despite the centrality of language for the development of children's biological knowledge, little research focuses specifically on the biological concepts in children who are impoverished in language, like children with Autism Spectrum Disorders (ASD). The research in this population might be particularly helpful in determining the actual role of language beside cognition in the emergence of biological concepts. Due to their communicative impairments, these children might lose important opportunities to learn from natural conversations initiated by caregivers about food, who point out that substances which appear edible may in reality be inedible because they have been contaminated. Thus, if language plays a major role, children with ASD might be at risk of not developing a sense of contamination for food and related disease-avoidant behaviour.

The knowledge about food contamination is of particular interest for children with ASD, in the light of the protracted debate over the role of gastrointestinal (GI) issues in autistic symptoms. The greatest interest among families focuses on the possibility that GI problems might play a role in the etiology of autism. Specifically, autistic symptoms are thought to be linked with inflamed gut, which prevents them from properly digesting the proteins found in food such as milk and bread. Consequently these proteins (peptides with opioid activity derived from foods which contain gluten and casein), flow into the bloodstream and “switch-off” part of the brain, causing or aggravating the symptoms of autism (Wakefield, Murch, Anthony, Linnell, Casson, et al., 1998).

On the basis of this view, a growing number of families tend to place their children with ASD on specific carbohydrate diets to cure gut inflammation, even though the results of these diets in terms of a reduction in autistic symptoms severity is far from scientifically proved (Elder, Shankar, Shuster, Theriaque, Burns, Sherrill, 2006). The common assumption is that these diets have no negative side effects, so there are no reasons to “leave no stone unturned’’(Elder, 2008; Green, 2007; Metz, Mulick, & Butter, 2005). Unfortunately, such assumption is not accurate. These diets require to the families to spend extra money to buy special foods and to dedicate extra time to prepare separate meals (Mulloy, Lang, O’Reilly, Sigafoos, Lancioni, Rispoli, 2010)”. Moreover, they might determine social stigmatization (e.g., because the child with ASD may not be able to eat the same foods as peers at a party) and they might be associated with an increased risk of nutritional deficiencies (Mulloy, Lang, O’Reilly, Sigafoos, Lancioni, Rispoli, 2010). Last but not least, there are a number of anecdotal reports of parents published online that describe as extremely expensive the process of putting a child into these diets, both in terms of consultations and in terms of biomedical analysis, which are necessary to start and to monitor the biological effects of these kind of treatments (e.g.:http://www.blogher.com/identifying-and-avoiding-autism-cults?page=0,1)”.

The results of the studies mentioned before and the anecdotal reports of the parents published online seems to indicate that, beside the lack of scientifically proved positive effects, these diets might sort some kind of negative side effects to the children’s health and to the quality of life of their family (burning considerable resources in terms of efforts, time and money) and therefore they need to be considered with great caution.

The only certain link between GI diseases and ASD “symptoms” at the moment is that GI symptoms seem to exacerbate behavioural problems in this population. Because of communication impairments, many GI symptoms in children with ASD present as behaviours like sleep problems, aggression and irritability. ASD children acting out may have pain or distress due to undiagnosed GI problems. The issue is not irrelevant if we consider that a variety of studies support a high frequency of GI complaints in this population (Valicenti-McDermott, 2006), even though the prevalence varies greatly from study to study due to the differences in groups evaluated and in the interpretation of GI symptoms assessed (Buie, 2011). For all these reasons, beside the lack of scientific evidences to support the role of GI problems in autism etiology, GI problems merit close consideration in children with ASD.

On the basis of these considerations, my Thesis aimed to study the role of language in contamination sensitivity, by investigating food rejection behaviour in children with ASD and its possible role in the prevalence of GI symptoms in these children. The introductory chapter (Chapter 1) illustrates the ontogenetic development of contamination sensitivity in typically developing children (TD) in relation to cognitive, affective and social abilities. Then, it describes the lack of contamination sensitivity in children with ASD and its possible implications for disease avoidant behaviours, particularly ones aimed at preventing GI disorders. Next, auditory processing deficits in individuals with ASD are illustrated, in the light of empirical evidence from several behavioural and neurological studies. This topic is of particular interest for my Thesis because, if language plays a major role on contamination sensitivity, then children with ASD might be at higher risk for not developing this ability due to their auditory processing deficits.

Finally, the roots of contamination sensitivity are described in terms of the intuitive perception of disgust. The use of nonverbal paradigms to evaluate implicit contamination sensitivity in children with language and social impairments is discussed, like eye tracking techniques and measures of variations in pupil size. Eye tracking provides a non invasive measure of where attention is spontaneously driven during the visual exploration of a stimulus. This technique has been successfully used in individuals with ASD to gain information about which aspects of the stimuli are favoured by participants, which in turn provides relevant insights into the underlying information processing (see for example Riby and Hancock, 2008).

The study of the variations in pupil size in response to visual stimuli provides a nonverbal measure of attention. Recent studies indicate that any stimuli that have some relevance to the observer are likely to provoke a pupillary response in the form of dilation, which are correlated with changes in activity in neurons of the locus coeruleus (Rajkowki, Majczynski, Clayton, and Aston-Jones, 2004). The locus coeruleus is a subcortical brain structure that constitutes the hub of the brain's noradrenergic system

(Aston-Jones, and Cohen, 2005). One current hypothesis is that the noradrenergic system, which originates in the locus coeruleus, mediates the functional integration of the attentional brain system. The connection between pupil diameter, the locus coeruleus and the noradrenergic system allows researchers to track changes in attention by the means of variations in pupil size (Laeng, Sirois, and Gredeback, 2012).

The following chapters illustrate the aims, the methods and the results of a series of studies, developed to investigate the role of language on contamination sensitivity in children with ASD. Five research aims were considered, which correspond to five research questions still open in the literature: 1) Are children with ASD, who are impoverished in language and communication, at a major risk to develop a reduced sense of contamination compared to typically developing children? 2) Is a lack of contamination sensitivity in children with ASD related to GI problems? 3) If there is a subgroup of children with ASD that lack contamination sensitivity, would it be significantly lower in auditory processing skills compared to the children (ASD and TD) with contamination sensitivity? 4) Is an explicit reaction to a contaminant based on an implicit sense of contamination? 5) Does a linguistic information about contamination (like telling children that an insect is disgusting) affect implicit contamination sensitivity in ASD?

These questions were addressed in four Studies. In Study 1 we replicated Kalyva, Pellizzoni, Tavano, Iannello, and Siegal (2009), in which contamination sensitivity was investigated for the first time in ASD, in comparison to TD children and children with Down Syndrome (DS). A reduced sense of contamination in ASD children was found, while the majority of TD and children with DS refused to drink a juice that had been contaminated with a cockroach. In line with Kalyva's et al. study, our results confirmed a lack of contamination sensitivity in almost half of children with ASDs but not in TD and DS children. As the ASD and DS children did not differ in verbal and nonverbal mental age, the results point to a detrimental effect of impoverishment in language and communication on the development of contamination sensitivity. In Study 2, we established that a lack of contamination sensitivity in ASD is linked to a deficit in auditory processing abilities, which were lower in children with ASD who were willing to drink a contaminated juice.

However, these two studies were limited in that they involved only one measure of contamination sensitivity, which was a measure from a behavioural task in which the experimenter asked a child if it was OK or not to drink a juice that had been in contact with a cockroach. The use of behavioural tasks to test contamination sensitivity in clinical population, like children with ASD, is quite new in the field. It is possible that the linguistic and the social demands involved in these tasks might mask to some extent the actual potential of these individuals to be sensitive to contaminants and contamination. Moreover, the task requires the children to express a judgement on the quality of the juice (if it is drinkable or not), which in turns requires some executive control. In order to refuse the contaminated juice, the child has to inhibit the prepotent response to drink it on the basis of the knowledge that contamination occurred. Since children with ASD are known to be impaired in executive functioning (Hill, 2004), their tendency to drink a contaminated juice might be due to a deficit in inhibition rather than an actual lack of contamination sensitivity.

With these considerations in mind, in Study 3 and Study 4 we paired the behavioural evaluation of contamination sensitivity with implicit measures, like preferential looking toward a contaminated *vs* an uncontaminated drink and variations in pupil size in response to contaminants. The stimuli developed for the purposes of these studies were videos in which we re-created a behavioural contamination task in a sequence of actions organized as follows: 1) an adult poured some juice into two glasses; 2) an insect floated on top of the juice in one of the two glasses and it was removed without a trace; 3) then the adult asked the child “Which one is for you?”. The videos were presented to the participants whilst measures of preferential looking toward the two glasses (contaminated *vs* uncontaminated) and variations in pupil size were considered. Preferential looking as implicit index of “choice” between the two glasses was used, in order to avoid any interference due to social and communicative impairments in participants with ASD. Measures of pupil size as involuntary reactions to contaminants were used to eliminate possible interference from executive control deficits in children with ASD, required by voluntary choices between two options (contaminated *vs* uncontaminated glass), both at the verbal and nonverbal level.

In Study 3 we found that TD children and children with ASD possessing contamination sensitivity had a looking preference for an uncontaminated drink in contrast to children with ASD who did not possess contamination sensitivity. Moreover, children with ASD who lacked contamination sensitivity didn’t show a pupillary response to a contaminant (a cockroach floating on top of a glass of juice), while TD children and children with ASD with contamination sensitivity showed pupil dilation right after the stimulus onset. These results seems to indicate that in children with ASD who lack contamination sensitivity, the learning process about contamination and disgust might have been compromised not only at a semantic level but even at an implicit one.

In Study 4, we investigated the effect of language on contamination sensitivity by adding informative linguistic messages (like “Oh, there is a cockroach, it's disgusting!”) to the videos used in Study 3. When we measured the effect of language on the variation of pupil size in response to a contaminant, the results showed that children with ASD who lacked contamination sensitivity also showed an increase in attention to the insect, even though to a lesser extent compared to TD children and ASD children with contamination sensitivity. The results demonstrated an effect of language on implicit sensitivity to disgust elicitors in TD children and ASD children with contamination sensitivity but not in ASD children who lack contamination sensitivity. This effect, very subliminal and not actually linked to an explicit level of understanding of hygiene and contamination, might be a window to teach new information about contaminants to these children.

The role of contamination sensitivity in GI problems in ASD children have been addressed in Study 2, Study 3 and Study 4. Basically, ASD children in our samples did not differ from controls in the prevalence of GI symptoms and there were no significant differences in attitude toward disgust and contamination in their parents, as measured by the Disgust Sensitivity Scale (Haidt, MacCauley, and Rozin, 1994). However, ASD children who lacked contamination sensitivity were perceived as less aware about contamination by their parents than the children in the other two groups.

The results of the four studies are summarized and discussed, in terms of strengths and weakness, in the last chapter of the Thesis. The findings of each study answer to some extent to the research questions still open in literature, illustrated in Chapter 1. The results of this Thesis are quite new in the field because little is known in regard to the role of language in the development of biological concepts in children with ASD, particularly in the realm of disgust and contamination sensitivity. Moreover, the use of a nonverbal paradigm leads to new possibilities in exploring biological knowledge in these children, despite their deficits in language and communication.

Some possible limitations of the studies are considered, like insufficient consideration of possible mediators of access to language and conversation about food and contamination in daily life in ASD children. Even though these children might acquire a rich vocabulary, they are severely impaired in the ability to “map” the meaning of the words in a natural social interaction by using a number of social cues, like the direction of the speaker’s gaze (Baron-Cohen, Baldwin, and Crowson, 1997), gestures and facial expressions (Parish-Morris, Hennon, Hirsh-Pasek, Golinkoff, and Tager-Flusberg, 2007). This ability might play a major role in the cultural transmission of knowledge about contamination in ASD.

Finally, future directions of research are outlined, like the possibility of investigating disgust in other populations of individuals impoverished in language, like preverbal infants or deaf children. Moreover, it might be of interest to develop an intervention study to implement a sense of contamination in children with ASD. Such intervention could promote the independence of these children in regard to disease-avoidant behaviour, helping their well being and that of their family.

1.1 The development of disgust in children

Despite the ability to treat and cure a number of acute and chronic diseases, humans are still vulnerable nowadays to a number of contagion diseases. For this reason, individuals need to learn and to adopt effective disease-avoidance behaviours, to protect themselves from infections very early on in development. People who are aware of the most important disease threats are likely to actively interrupt the chain of transmission of infective diseases, by reducing the possible opportunities of contagion.

However, the translation from “awareness” to “action” is not automatic, but it seems to be mediated by cognitive, affective and socio-linguistic processes. Specific cognitive abilities, like categorization and the understanding of casual relations, predispose individuals to recognize the perceptual cues that indicate the presence of a contaminant in the environment and to link it with the biological concepts that connote disease (Faulkner, Schaller, Park, and Duncan, 2004). Affective reactions belonging mainly to the domain of disgust seem to trigger the behaviour of individuals to avoid contagion. Disgust is a food rejection emotion characterized by revulsion at the idea of ingestion of harmful substances, such as poison, waste products of human and animal bodies or contaminated food (Angyal, 1941; Fallon and Rozin, 1983). This universal and basic emotion (Ekman, 1992), that prevents humans from ingesting dangerous substances like poisons or food that has been contaminated by noxious microorganisms, has its roots in distaste, an innate rejection of bitter-tasting substances already present in new-borns (Steiner, 1979).

However, besides its biological grounds, disgust evolves in very sophisticated and ideological forms through a process of basic disgust socialization from parents and other significant members of the community. It is through the mechanism of social referencing that children learn how to recognize and react to a number of disgust elicitors. Moreover, in the course of natural conversations, children are instructed about food, non-food and contamination by a process of generalization from universal primal disgusting entities (Rozin and Fallon, 1987).

In summary, the behaviour of individuals toward contamination and illness seems to be grounded in both biological predispositions and in a combination of cognitive, affective and socio-cultural abilities, which progressively emerge in childhood.

Along with its adaptive biological function, which is to prevent humans ingesting noxious substances like poisons or food that has been contaminated by harmful microorganisms, disgust also serves a fundamental cultural function, which is the transmission of important social values (Rozin, 1982). In fact, the acquisition of disgust is a special case of the acquisition of culture values on the basis of a constant interaction between affect and cognition. A number of disgust elicitors are avoided because of their strong negative affective intrinsic properties, rather than for their actual health reasons. Disgust seems to be learned from the behaviour of parents and other adults toward disgusting substances, thanks to the mechanism of social reference and throughout language and communication (Rozin, 1982). Observations of interactions between children and their parents indicate that from as early as 14 months, there is an appreciation by children that disgusting substances have special significance for adults (Dunn, 1986). There is also a great deal of talking between children and parents about this subject (Dunn, 1986).

Evidence from feral children and from ontogenetic development confirm that the emergence of disgust requires some degree of enculturation. An examination of the case studies of 50 feral children show that all of those children had food preferences during isolation from human contact and that they showed evidence of rejection on the basis of distaste, danger and inappropriateness. However, none of them showed any evidence of disgust or any sense of contamination (Malson, 1972). Furthermore, evidence from food preference studies on young children show that disgust emerges quite late, sometimes between 4 and 8 years of age (Rozin, Hammer, Oster, Horowitz, and Marmora, 1986; Rozin, Fallon, and Augustoni-Ziskind, 1986), while distaste is present in newborns.

The ideational ground of disgust makes it different from other forms of food rejection, like distaste and danger, which are merely motivated respectively by sensory factors, usually taste or odour, or by anticipated harmful consequences. Conversely, disgust is the rejection of a substance on the basis of its origin or nature. The majority of disgust elicitors are indeed animal products or features that humans share with animals: excreting, sex, soft body interiors and death (Rozin, Haidt, and McCauley, 2000). Since in the greater numbers of cultures, humans display a strong desire to be distinct from other animals, disgust serves the cultural and social function to help humans be different from animals in their habits and in their interpersonal behaviours (Rozin, Haidt, and McCauley, 2008).

The psychological grounding of disgust is also highlighted by a special property of disgust elicitors, called "contamination": that is, the power to render an otherwise edible food unacceptable by mere contact. Contamination can be explained by two laws of "sympathetic magic", called "contagion" and "similarity", which are typical of traditional cultures but play an important role also in modern societies. Contagion can be summarized as "once in contact, always in contact" (Frazer, 1959), which means that things which have once been in contact with each other continue ever afterwards to act on each other. Similarity can be summarized as "like produces like" (Frazer, 1959), which means that resemblance in some properties indicates a fundamental similarity or identity. Rozin, Millman, and Nemeroff (1986) demonstrated empirically that the phenomenon of "contagion" operates in the domain of disgust, simply by dropping a dead, sterilized cockroach into a glass of juice and then removing it. Subjects found this juice much less desirable than a different type of juice, which contacted an innocuous object for the same period of time. Rozin et al. (1986) also demonstrated experimentally the phenomenon of "similarity", showing that subjects preferred to a large extent to consume a piece of chocolate fudge shaped as a muffin, as opposed to a piece of the same fudge shaped as dog faeces.

Along with an operational definition of disgust, Rozin delineated a developmental sequence of disgust and contamination sensitivity, exploring the conception of food in three group of children, aged between 3 and 12 years old, and a group of adults (Fallon, Rozin, and Pliner, 1984). Participants were interviewed about their individual motivations for not ingesting substances, using as a frame of reference the adults' taxonomy of non-foods (Fallon and Rozin, 1983): distaste, danger, inappropriateness and disgust. Their reactions to stories involving contamination of liked foods by various substances were evaluated, employing a judgment task followed by interviews modelled on general Piagetian techniques. The results showed a developmental sequence of the categories of rejection: distaste (rejection based purely on sensory characteristics) emerged first; danger appears next (rejection based on anticipated harm following ingestion); the ideational type of rejection (rejection based on the idea of what something is or where it comes from) was the last to appear and it was differentiated into disgust (affective rejections to substances that became offensive) and inappropriateness (neutral rejections of substances as simply not food).

Contamination sensitivity (the perception that disgusting substances render a liked food inedible by contact or for ideational association) was not present in younger children but it appeared gradually with age. Younger children were indifferent to contamination, believing that the removal of the contaminant was sufficient to return the beverage to its original state. Although the study of Fallon et al. (1984) didn't actually investigate the underlying mechanism behind the emergence of the different categories of disgust, it was claimed that young children do not show a "contamination response" because they are unaware of the physical chemistry of solutions (e.g., diffusion and its lack of reversibility), due to a lack of cognitive ability or a failure in education. It is possible that they misapplied the principle of reversibility, that is, they believed that "diffusion" is reversed by the removal procedure. This could have been prompted by the visually salient fact that a contaminating substance, apparently identical to what fell into the juice, was removed. This is consistent with Piaget's argument that preoperational children consider the world is it appears to be, no matter what the reality. However, the age of appearance of these concepts might have been underestimated in this study because the children were interviewed mainly with verbal report, that might be a less sensitive measure of conceptual attainment than judgmental responses.

To confirm the results of Fallon et al. (1984) study under more reliable experimental conditions, Rozin, Fallon, and Augustoni-Ziskind (1986) evaluated the responses of children aged from 3.5 to 12.5 years to the actual contamination of liked food by adding disgusting items to it. They also extended the range of disgusting items used before. The results were consistent with those obtained previously (Fallon et al., 1984): younger children, aged between 3 and 6 years, showed less contamination sensitivity than did older children and adults. However, because the actual mechanisms involved in contamination sensitivity were not specifically investigated, it was still unclear whether the younger children's tendency to consume contaminated food represents a lack of the cognitive abilities necessary to conceptualise contamination or the incomplete emergence of an ideational based category of disgust.

These results were confirmed and extended by Rozin and colleagues in another empirical study (Rozin, Hammer, Oster, Horowiz, and Marmora, 1986), that explored the acceptance of a series of disgusting substances from adult rejection categories in children aged between 16 month and 5 yrs. The majority of children under the age of 2 accepted to put almost anything into their mouth, while the incidence of rejection of adult substances rises markedly after 2 years of age. These results confirmed that distaste is the central category of rejection until the child develops rejection based on danger and, later, disgust (Fallon et al., 1984). Moreover, they highlight the socio-cultural nature of disgust, since they demonstrated that much of what children learn in the second year of life is what not to eat.

Taken together, these studies seems to confirm that disgust develops late in childhood, thanks to the maturation of specific cognitive abilities that allow children to understand the biological basis of contamination and throughout a process of education, that enables children to distinguish between edible and inedible food, and to identify cultural specific characteristics of food that has to be rejected.

1.1.1 Contamination sensitivity as a general aspect of cognitive development

However, a number of succeeding studies found that even younger children, between the age of 4 and 5, show contamination sensitivity. In 1988, Siegal demonstrated that preschoolers display some knowledge of contagion and contamination, that might be explained in terms of children's ability to understand causal relations. Preschool children, grade 1 and grade 3, were required to evaluate other's explanations for illness, to indicate the likelihood that illness would occur, and to predict their own preventive health behaviour. Specifically, children were shown videotaped segments of puppets with colds and toothaches who explained their conditions in terms of contagion and immanent justice. The term "immanent justice" refers to the belief that illness may be regarded as a form of punishment that inevitably follows a sin or a transgression (Piaget, 1932). The children were instructed to evaluate and correct the puppets' explanations and, in addition, to indicate the possible effects on health of drinking milk that had come into contact with contaminants. The results showed that even preschoolers demonstrated the same knowledge of contagion and contamination as older children. However, compared to the 3rd graders, younger children were less likely to reject proximity to a sick person and to reject naughty behaviour as cause of toothaches. Moreover, preschoolers rejected the proposition that an ailment caused by an accident is contagious and they also accepted that contamination through contact with a dirty spoon can be prevented by washing. Altogether, the results of this study showed that preschoolers have a more substantial knowledge of contagion than has been estimated by previous studies. This divergence might be explained in terms of methodological issues inherent in the experimental procedure used in previous studies. In particular, direct and prolonged questions are known to underestimate the actual knowledge of children (Gelman, Meck, and Merkin, 1986). Moreover, even though children might know that a drink that has been in contact with a foreign object may be harmful in everyday situations, they may not be aware that an adult might offer children contaminated food to test their understanding of concept of contamination (Siegal, 1988). Thus, rather than lacking conceptual competences in their knowledge of contamination, children might simply have misinterpreted the procedural requirements of the testing situation used in previous studies.

These finding were extended in 1990, in a study showing that preschoolers have the prerequisites to understand the concept of contamination (Siegal and Share, 1990). It was proposed that, in order to succeed on contamination tasks, whose clear goal is the avoidance of the ingestion of non contaminated food, children must distinguish between appearance and reality. In particular, children have to ignore the appearance of the food and focus on the real state of the substances in order to decide to eat them or not. Children’s identification of a liquid that contained invisible contaminants and their appreciation of the real goodness of an apparently edible substance were tested in very young children, aged between 3 and 4. To examine contamination sensitivity, participants were offered juice that had been in contact with an insect. Most children indicated that the juice was not good to drink even though the cockroach had been removed. Moreover, they made accurate evaluations of others’ responses to this type of situation and they were able to predict the possible actions that would protect others. To evaluate the importance of the appearance/reality distinction in contamination sensitivity, participants were presented with an apparently edible food that was in reality mouldy (a piece of mouldy bread covered by a breakfast spread). In contrast to a control group that received fresh bread without mould, most children responded that the bread would not be good to eat even after the mould was concealed by the spread. Having seen the mould, these children identified the bread with vegemite, apparently safe, as not good to eat. These results demonstrated that the representation of appearance and reality, usually absent in children at this age, may be particularly advanced in relation to familiar and salient situations like ones concerning food. The understanding of appearance/reality assessed by this task doesn't need to be necessary consciously accessible to the children, but it could be just implicitly represented in their behaviour as a means to avoid the ingestion of contaminated food.

Subsequent researchers not only confirmed that preschoolers have some knowledge of contamination but they also investigated the role of specific cognitive abilities in the emergence of contamination sensitivity at this age. In particular, Au, Sidle, and Rollins (1993) studied children's understanding of conservation of matter as a possible basis for children's contamination awareness. The concept of "conservation of matter", which states that matter can neither be created nor destroyed but it continues to exist, even after it becomes invisible after dissolving in a liquid, is based on the belief that materials are made up of tiny particles. According to Piaget's view, children begin to appreciate that substances are made up of tiny particles by age 8 or 9. However, classical Piagetian studies investigated children's ability to understand that dissolved sugar continues to taste sweet and have weight after dissolution, requiring the children to know how a balance scale works and what it measures. It is possible that the classical tasks were too confusing and demanding to allow the children to respond appropriately. To avoid these kinds of methodological issues, Au and colleagues (1993) devised a revised procedure to test if children under age 8 could demonstrate some understanding of "Piagetian atomism" under more favourable conditions, which included a discussion about the taste of sugar and water before the taste conservation questions. Moreover, the test questions about conservation of matter were rephrased in terms of a long list of familiar daily activities to help children to make sense of the questions.

This revised procedure was successful in revealing young children's belief that a substance can continue to exist and maintain its inherent properties, such as taste and weight, even after it has become invisible. By the age of 3 years, some children could appreciate conservation of matter and the existence of invisible particles despite visual disappearance. In addition, they could make use of the particle concept to come up with a plausible mechanism for how a substance can continue to exist and maintain its inherent properties even after it becomes invisible upon dissolution. The proportion of children who could do so increased with age (Au et al., 1993). Children's contamination awareness was tested with stories about a chunk of contaminant falling into a glass of the child’s favourite juice. Each child was then prompted to consider the possibility of tiny, invisible bits of the contaminant remaining in the drink, after the chunk was taken out. After the prompt, the child was asked to give and justify a new rating for the contaminated drink. The results confirmed that, as shown in previous studies, contamination awareness increases with age. Moreover, the majority of children by age 5 can already appreciate that contaminants can exist as tiny, invisible particles and can thereby have effects, like causing illness, even though the individual particles are too small to see. Although young children tend not to use this concept spontaneously in deciding whether something is contaminated and in justifying their decision, most 5 – 7 years olds would do so once they are asked to consider whether tiny invisible contaminant particles may have stayed inside the drink. Early knowledge of contamination was confirmed also by a study by Kalish (1997), which found that young children are able to distinguish between mental and bodily reactions to contamination. Children aged between 3 and 5 distinguish reactions mediated by representations from those mediated by physical interactions. In particular, they were aware that knowledge determined mental reactions to contamination while physical contact determines bodily reactions. Children also judged that emotions and illness were unintentional, as they were seen as beyond conscious mental control. These results indicate that children don't use intentions to distinguish between psychological and physical reactions to contamination. Both mental and bodily reactions may be involuntary. However, when questioned about particulars of emotional and illness reactions, most preschoolers did not realize that illness takes time to develop. These data suggest that, even though preschoolers do distinguish between physical and mental reactions to contamination, they have a poor understanding of the actual bodily processes involved in illness. Rozin and other authors also explored a more advanced level of contamination sensitivity: the understanding of contagion as a result of associational contamination thinking. “Associational contamination” is an associational form of contamination: an acceptable food may be rejected because it is associated with the contaminating substance in the absence of any physical contact with the contaminant (Fallon, Rozin, and Pliner, 1984). For example, most adults would refuse to drink a liked beverage from a brand new dog bowl or to eat their preferred soup if it has been stirred with a brand new comb (Fallon et al., 1984; Rozin, Fallon, & Mandell, 1984). Associational contamination differs from trace contamination, in which the actual physical contact with a disgusting substance renders an otherwise acceptable food inedible (Fallon et al., 1984; Rozin & Fallon, 1981). Trace contamination occurs even if the contaminating substance leaves only a microscopic physical trace that cannot be perceived through taste or sight (Rozin & Fallon, 1985; Rozin, Fallon, and Augustoni-Ziskind, 1985). Rozin, Fallon, and Mandell (1984) showed that, while most adults show both trace contamination and associational contamination, young children seldom engage in these kind of thinking. Specifically, trace contamination responses do not appear in most children until the age of 7 or older and that associational contamination is rare before age 12.

However, these conclusions were based on responses to illustrated stories rather than to real events. Therefore, it is not possible to rule out the possibility that younger children's actual behavior might differ from their prevision, triggered by a story of how much they would like various contaminated beverages.

A subsequent study (Rozin, Fallon, and Augustoni-Ziskind, 1985) considered children's responses to the actual contamination of a liked beverage by adding disgusting items to it, like insects or human hair. The degree of contamination in the tasks was manipulated into three levels: no contamination, trace contamination and associational contamination. Prior to pouring the juice, the experimenter combed her hair and then returned the comb into a case. The experimenter poured apple juice into a glass and removed a different comb from a clear plastic package. She then said, "This is a brand new comb that I bought yesterday, all washed and cleaned, I am going to stir your juice with this comb." Then, she stirred the juice and asked to the child: "Will you drink some juice?". After that, the experimenter took another comb from another case and said, "This is a comb that I used to use every day before I went to bed and when I got up in the morning. All washed and clean. "Then, she stirred the juice with this comb and asked to the child "Will you drink some juice?". Finally, she pulled out the comb that she had previously used to brush her hair. This comb was actually a clean duplicate of the one she had previously used to comb her hair, taken from a second compartment in the same comb case. She now stirred the juice with this apparently used and unclean comb and asked to the child "Will you drink some juice now that I have stirred the juice with this comb?".

The results confirmed the prior findings as to the age of onset of both types of contamination sensitivity and extended the range of contaminating substances studied to include human residues (in this case, hair). The author claimed that the onset of contamination responses may be influenced both by the achievement of a requisite level of cognitive development (the understanding of some basic physical principles, such as diffusion in solutions) and by the prior establishment of a category of "disgust."

Springer and Belk (1994) criticized Rozin’s study, pointing out that children might have assumed that the beverage was drinkable under the effect of a mild social pressure, namely because an adult offered it. Rather than lacking the knowledge that a drink that has been in contact with a foreign object may be harmful, the children may have simply misinterpreted the requirements of the situation. Specifically, they may not believe that an adult might offer children a contaminated drink in the effort to test their understanding of the cause of illness. To verify this hypothesis, Springer and Belk (1994) rephrased the question asking children to predict whether a story character would feel sick after drinking a beverage, who’s proximity with a contaminant was systematically manipulated. The findings showed associational contamination thinking among 40% of 3 and 4 years olds. Most 7 and 8 years old recognized the need for physical contact between the bug and the juice to render the juice inedible. However, since for some children the mere presence of a contaminant renders a physically removed substance harmful, it was clear that sometimes the associational contamination is involved in children’s reasoning.

However, it is important to consider that Springer and Belk (1994) observed associational contamination thinking in young children by examining an insect as a contaminant. So it was not clear from their study whether or not young children associated contamination with particular types of contaminants such as dangerous or disgusting ones. Moreover, in previous studies about associational contamination, participants may have implicitly assumed the existence of actual physical traces of contaminants. Specifically, they may have believed that the comb and flyswatter had germs (Rozin, Fallon, and Augustoni-Ziskind, 1985) or that washing with soap did not destroy every trace of germs in the faeces (Fallon, Rozin and Pillner, 1984). So the actual mechanisms of associational thinking needed to be specified and further investigated.

In line with these studies, Toyama (1999) examined whether an assumption of physical traces would lead to associational contamination thinking. College students, 4 and 7 years old children were presented with stories about disgusting, dangerous and taste-based contaminants. In one story, the contaminants were placed in a beverage, while in other stories they were placed into either an uncovered or covered beverage. Participants had to evaluate whether contamination would occur. If associational contamination thinking was based on feelings of disgust or fear, people would mention associational contamination for disgusting and dangerous but not for taste-based contaminants, even if the contaminants were placed next to a covered glass (so it could not possibly leave traces in the beverage).

The results delineated a developmental sequence of associational contamination reasoning. Preschoolers seems to understand the nature of contagion by mere physical contact and their tendency to rely on perceptual clues plays an important role in their associational thinking. When contaminants left invisible traces, young children were more accurate in using physical principles of contamination, and their performance in predicting contamination became similar to adult's performance. Conversely, college students and 7 years old children were engaged in thinking about contamination more often for disgusting and dangerous items than for taste-based contaminants. In summary, perceptual cues were more important for young children than for adults and older children in this kind of reasoning about food and contamination.

Recently, contamination sensitivity has been investigated again as part of a more general biological understanding of illness and contagion, in an effort to define universalities and differences in the mental schemes that children develop to categorize the variety of biological phenomenon. Raman and Gelman (2005) found that preschoolers not only have a knowledge of contamination but that they also recognize that not all disorders are transmitted exclusively through germ contagion. In particular, they were able to understand that certain disorders, like allergies, are transmitted by birth parents, thus they distinguish between inheritance and contagion as sources of disease. Moreover, this study investigated the cues that preschoolers and adults use to differentiate between genetic disorders and contagious illness. In the presence of kinship cues, children distinguished genetic disorders from contagious illness, while in the presence of contagion cues, preschoolers selectively applied contagion links primarily to contagious illness. With novel illnesses, preschoolers and adults inferred that permanent illnesses were more likely to be transmitted by birth parents than by contagion. These results suggest that by the preschool years, children demonstrate a rather advanced biological understanding of contagion and illness.

These results were extended by another study, which investigated the impact of psychosocial factors in the transmission of contagious illness, injuries, and disgust (Raman and Gelman, 2008). Participants, ranging from preschoolers through to adults, judged the likelihood that a character would get sick after being contaminated by another individual that was not in any relation to the character, a disliked person, a best friend or a family member. The effects of psychosocial relatedness on judgements of disgust were evaluated, along with the influence of the knowledge of germs on judgements of disgust. Overall, preschoolers through to 2nd graders judged that any type of relatedness decreased the possibility of contracting illness from another person. However, relatedness had no effect on judgements of injury transmission. These results suggest that young children treat the psychological and biological domains as distinct but mutually interacting (Raman and Gelman, 2008).

Recently, Legare, Wellman, and Gelman (2009) advanced what is known about the development of contamination concepts by re-examining children's explanations and predictions for the biological phenomenon of contamination. Preschoolers and adults heard vignettes concerning contamination and they were asked to predict or to provide an explanation of the specific phenomenon described in there. Even very young preschoolers gave explanations based on contamination sensitivity. Most children indicated an invisible mechanism, like germs, as a possible explanatory mechanism for contamination. Children were significantly more accurate with their explanations than with their predictions, while adults performed at ceiling across both explanation and prediction tasks. The effect of desirability of the contaminated substances on contamination sensitivity was also investigated. Although desirability affected responses, participants were more accurate on explanation than prediction questions. In general, these results demonstrated a significant advantage for explanation in children's reasoning in the domain of everyday biology (Legare et al., 2009), which suggests a possible crucial role for explanation in children's causal knowledge structures but also in the learning process for such causal knowledge.

Since explanations are more sophisticated than predictions, they might provide an important base for further learning. Considering that explanations involve theoretical unobservable elements to explain phenomena, they engage children in the important interplay between data and theory that leads to theory change. The claim that explanations may play an important role in children's learning is consistent with the results of other studies, which demonstrated that requiring children to explain events enhances learning over simple feedback about the correctness of their predictions (Amsterlaw and Wellman, 2006; Siegler, 1995). Once again, the study of naïve biology was a powerful mean to better understand the structure of children's cognition and the possible implication of specific cognitive functions in the process of learning.

1.1.2 Contamination sensitivity as a result of cultural influence

The empirical findings reviewed so far highlight the tendency of a consistent number of researchers to conceptualise the development of contamination sensitivity as a general aspect of cognitive maturation rather than as a result of specific learning about the properties of food.

Conversely, another body of studies focuses on the actual influence of social and cultural mechanism in promoting the emergence of the ideational property of disgust. In particular, these studies investigate how children learn the affective and ideational characteristics of disgust elicitors, to what extent cultural influences shape the children's behaviour about food and illness and how it comes to be that children develop associational contamination thinking, that becomes more and more abstract with development since it gets generalized to moral behaviour.

In 1984, Rozin, Fallon, and Mandell explored similarities in attitudes to food, especially sensitivity to cleanliness and contamination of foods, as well as food preferences, between young adults and their parents. The hypothesis was that parent-child resemblance in disgust sensitivity could result from specific childhood socialization experiences, in which parents influence their children's food attitudes. Rozin suggested that it is reasonable to believe that each family teaches its own interpretation of the cultural view about food, regulating the child's exposure to different foods and displaying attitudes to food for the child to imitate. The results confirmed this hypothesis, finding an effect of family influence on disgust perception about food.

However, these results were challenged by a recent study, which focused on "socially mediated rejection" (Toyama, 2000), that is the tendency to consider a food inedible not only in the physical but also in the social sense. For example, people sometimes drop food at mealtimes and, once dropped, the food becomes dirty even without physical contact with contaminants and people may not eat it, especially in some social contexts like restaurants. Children aged between 1 year and 4 years were observed in two contexts, at home and at school, and it was found that even 2 year olds reacted differently at fallen food at home (they almost always ate the fallen food) and at school (they seldom ate the food after it touched the floor). Moreover, participants were asked to predict a story character's bodily and emotional reactions to eating fallen food. Preschoolers were able to specify that physically contaminated food would cause bodily harm and that social contexts do not determine physical reactions to food and germs. In spite of such early awareness of physical principles of contamination, information from adults was confusing to children. Mothers and teachers often the say and do things that contradict physical principles. Given such confusing information about edible/inedible distinction derived from caretakers, preschooler's sophisticated understanding of physical contamination is quite surprising, and it challenges the hypothesis that children acquire these beliefs from exposure to information in the social world.

Another way to study the influence of social and communication factors in the development of disgust is to compare how intuitive concepts of contamination are manifested in children living in different countries.

Hejmadi, Rozin, and Siegal (2004) compared American and Hindu Indian children's responses to situations of potential contamination and purification. In Hindu India, food is the major vehicle for maintaining social distinctions and provides the basis to develop a number of moral beliefs (Appadurai, 1981). A number of social rules govern the behaviour of the people toward food and, very often, rule variations are viewed as disgusting. Hence, the social and culture values about food might contribute to form a sense of contamination sensitivity in Hindi children that might differ from those of children raised in Western countries. The results showed a considerable similarity in the development of contamination sensitivity in Indian and American children, except that Indian children responded significantly more strongly to stranger or cockroach contamination and, with increasing age, viewed contamination as more impervious to any kind of purification. These results might be explained in terms of cultural differences, given that hygienic and social rules about food are so relevant in India.

Recently, Stevenson, Oaten, Case, Rephacholi, and Wagland (2010) reinvestigated when and how different disgust elicitors are acquired, asking parents of children ranged between 0 to 18 months to rate how their child would react to 22 disgust elicitors. Different developmental patterns were identified for core, animal and sociomoral elicitors, with core elicitors emerging first. Then, children aged between 2 and 16 months were exposed alone and then with their parents to a range of elicitors, and self-reports, behavioural and facial expression data were obtained along with measures of contagion, conservation and contamination. The results supported the developmental model described by Rozin in the early ‘80s and its interpretation in terms of evolutionary functions, in which core disgust responses are acquired early to promote avoidance of pathogens.

Evidence for parent-child transmission were also observed, with parents of younger children emoting more disgust to their offspring and showing greater behavioural avoidance. Moreover, children’s reactivity to animal and sociomoral elicitors and contamination correlated with parental responsiveness. However, this was a correlational study, so the actual role of culture and socialization in the development of an adult-like sense of disgust needs to be further investigated.

1.2 *The lack of disgust in ASD and GI symptoms in this population*

Studies looking at neurological disorders or in individuals with brain lesions often help to clarify the neurological basis of specific processes. Very few neuroscience studies have explored neural responses to disgust elicitors and to facial expressions of disgust in neurological disorders. One study found that individuals with Huntington’s disease show a specific deficit in recognizing disgust faces (Sprengelmeyer, Young, Calder et al., 1996). Huntington’s disease is commonly seen as a basal ganglia disorder, characterized by abnormal movements, psychiatric problems and cognitive impairment. The results of this study revealed a marked deficit in recognizing disgust, which was significantly larger than the deficit observed for recognition of other emotions, like fear. Nearly half of all participants tested never used the emotion label disgust to describe any of the presented facial expressions. These results were confirmed by a following study, that indicated selective deficits in recognizing disgust in Huntington's disease (Sprengelmeyer, Young, Sprengelmeyer, Calder, Rowland, and Perret, 1997) and helped to define a possible neural substrate of disgust processing. Some brain areas, namely the anterior insula, some basal ganglia structures and some parts of the frontal cortex, are involved in the experience of disgust (Phillips, Young, Senior, Brammer, Andrew et al., 1997). The sense of disgust seems to originate from a "disgust system" in the brain, which is triggered by language and communication.

In line with the number of behavioural studies previously described in this chapter, recent neurological studies seem to confirm the centrality of language and communication to promote disgust sensitivity. However, little research focuses specifically on the development of biological concepts in children who are impoverished in language, like children with ASD. The research in this population might be particularly helpful in determining the actual role of language besides cognition in the development of biological concept. Due to their communicative impairments, these children might lose important opportunities to learn from natural conversations initiated by caregivers about food, who point out that substances which appear edible may in reality be inedible because they have been contaminated.

Thus, if language plays a major role, children with ASD might be particularly at risk of not developing contamination sensitivity for food and related disease-avoidant behaviour. In line with this consideration, Kalyva, Pellizzoni, Tavano, Iannello and Siegal (2009) compared contamination sensitivity in ASD, Down syndrome (DS), and typically developing preschool children (TD). Children of the three groups were asked if they would like to drink liquids that had been contaminated by insects. The results showed that almost the 50% of children with ASD did not show contamination sensitivity, saying that they would want to drink the juice. These children did not differ significantly from the subgroup of those who did not want to drink the juice in verbal mental age and in Theory of Mind abilities. However, children with ASD who displayed contamination sensitivity were significantly older in their chronological age than those who did not. This pattern of results suggests that there is a developmental delay in reactions to contamination among children with ASD, at least in response to a strong contaminant such as a cockroach that had been in direct contact with a drink.

In the light of the protracted debate over the role of the GI system in autistic symptoms, the question whether children with ASD might be impaired in disgust and contamination sensitivity is of particular interest. The studies of the GI system in ASD started from anecdotal descriptions of GI symptoms in children with ASD such as gastritis, abdominal pain, food intolerance, chronic constipation, and diarrhoea and evolved toward a series of studies aimed at investigating whether children with ASD are particularly prone to such GI symptoms. Reflux esophagitis, chronic gastritis, and chronic doudenitis were found (Horvath, Papadimitriou, Rabsztyn, Drachenberg, and Tildon, 1999), as well as chronic ileocolonic lymphoid nodular hyperplasia and inflammation, referred to as ‘‘autistic enterocolitis’’ (Wakefield, Anthony, Murch, et al., 2000). The prevalence of GI problems in this clinical population needs to be considered in relation to the prevalence of GI problems in the general population. Loening-Baucke (1998) reported that in children aged between 4 to 7 years in the United Kingdom, gastroesophageal reflux disease is experienced by 1 in 4 children (Rudolph, Mazur, Liptak, Baker, Boyle, Colletti, et al., 2001), and food allergies are reported at a prevalence of 5-8% (Sampson, 1999). So, GI conditions should be expected with similar frequencies in the autistic population. Some retrospective studies found a relatively low prevalence in ASD, similar to the general population: Fombonne and Chakrabarti (2001) found a history of GI symptoms in 18.8% of 261 autistic children; Taylor et al. (2002) reported GI symptoms in the 17% of children with ASD; Molloy and Manning-Courtney (2003) found a prevalence of GI problems in 24% of autism population.

However, other studies showed higher prevalence of GI problems in individuals with ASD. Horvart and Perman (2002) found a prevalence of GI disturbance in 76% of a general autistic population. A high prevalence of GI symptoms in ASD has been found by Valicenti-McDermott (2006): 70% of the children with ASD had GI problems, compared with 42% of the children with other neurological conditions and 28% of the children without neurological impairments. These results suggest a higher frequency of GI issues in individual with a neurological disorders but also that the GI problems in autism are not simply related to nonspecific neurological dysfunctions but that they might be a specific comorbidity with ASD.

Very recently Nikolov, Bearss, Lettinga, Erickson, Rodowski et al. (2009) evaluated GI problems in a large sample of children with ASD (n=170). The findings showed that 39 (22.7%) were positive for GI problems. Those with GI problems were not different from participants without GI problems for demographic characteristics, measures of adaptive functioning, or autism symptom severity. Compared to children without GI problems, those with GI problems showed greater symptom severity on measures of irritability, anxiety, and social withdrawal. Those with GI problems were also less likely to respond to behavioural treatment. However, even though this study is the first that investigated in a systematic way the differences between children with

ASD with and without contamination sensitivity, it was limited because children with ASD accompanied by hyperactivity and serious behavioural problems, such as tantrums, aggression, and self-injury, were over-represented in the sample.

In summary, the prevalence of GI problems in ASD varies largely from study to study, probably because of the differences in groups evaluated and in the interpretation of the GI problems assessed (Buie, 2011), so the debate is still open and more research is needed to better define the extent of GI problems in ASD.

For the parents of autistic children with GI problems, the treatment and the prevention for such disorders is of great interest, whether or not these disorders are especially common in this population. Indeed, GI problems seem to significantly exacerbate behavioural problems and therefore need consideration. Because of communication impairments, many of the GI symptoms in children with ASD are present as behaviours like sleep problems, aggression and irritability. It seems that some children acting out may have pain or distress due to undiagnosed GI problems.

Moreover, according to the so called “opioid-excess hypothesis of autism”, autistic symptoms are the consequence of the incomplete breakdown and excessive absorption of peptides with opioid activity, which derive from foods which contain gluten and casein. This phenomenon disrupts a number of biochemical and neuroregulatory processes, causing the symptoms of autism (Wakefield, Murch, Anthony, Linnell, Casson, et al., 1998). Even though the link between inflammation of the gut and autistic symptoms is far from scientifically demonstrated, a growing number of families tend to place their children with ASD on specific carbohydrate diets to cure gut inflammation (Elder, Shankar, Shuster, Theriaque, Burns, Sherrill, 2006).

Unfortunately, these diets might sort some kind of negative side effects to the children’s health and to the quality of life of their family, consuming considerable resources in terms of energies, time and money. These diets require to the families to dedicate extra time to prepare separate meals for the children and to spend extra money to buy special foods (Mulloy, Lang, O’Reilly, Sigafoos, Lancioni, Rispoli, 2010)”. Moreover, putting a child into these diets might result sometimes in social stigmatization (e.g., because the child with ASD may not be able to eat the same foods as peers at school) and these diets might be associated with an increased risk of nutritional deficiencies (Mulloy, Lang, O’Reilly, Sigafoos, Lancioni, Rispoli, 2010). Last but not least, there are several anecdotal reports of parents published online that describe as extremely expensive the consultations and the biomedical analysis, necessary to start and to monitor the biological effects of these kind of treatments (e.g.:http://www.blogher.com/identifying-and-avoiding-autism-cults?page=0,1)".

For all these reasons, any new knowledge in the field is welcome, in particular anything about the role of possible mediators which might expose children with ASD to a greater risk of GI disorders, such as a lack of contamination sensitivity.

1.3 Auditory processing deficits in children with ASD

1.3.1. Speech perception and social orienting in ASD

Communicative and language impairment is one of the core symptoms of ASD, ranging from an almost complete absence of functional communication, to adequate linguistic knowledge but impairments in the use of language to communicate effectively in different social contexts (Tager-Flusberg, 1996). For the past decade, a number of studies have investigated possible cause and origins of these deficits. Among the various hypothesis, these linguistic and communicative impairments have come to be viewed as closely linked to deficits in auditory processing of speech. Therefore, a number of studies have investigated if and to what extent individuals with ASD perceive and process human language.

In TD children, speech discrimination and social interest in speech are central to the early development of language (Kuhl, 2000). Unfortunately, there is empirical evidence that individuals with ASD lack a preference for human speech and they are quite impaired in the ability to detect and discriminate language. Klin (1991) demonstrated, in a rather pioneering study, that children with autism are characterized by a lack of interest for voices and speech. A group of young children with autism, paired with a group of mentally retarded and normally developing controls, were tested for preferential listening responses to the child's mother's voice and to alternative non human sounds. The results showed no preferences for the mother's voice in autistic children, in contrast to a strong listening preference to speech of the controls. Such a lack of attention to speech in children with autism is not grounded on auditory deficits for non-speech sounds. Kemner, Verbaten, Cuperus, Camfferman, and van Engeland (1995), using Event-Related Potentials (ERP), an electrophysiological technique often used to investigate auditory processing, found a prominent P3 (a component elicited in the process of decision making) to pitch changes in a stream of non-speech sounds.

Ceponiene, Lepisto, Shestakova, Vanhala, Alku, Naatanen, and Yaguchi (2003) investigated further auditory processing in ASD, with the hypothesis that, because socially meaningful stimulus events (like vowels) are physically complex, a deficiency in sensory processing of complex stimuli might contribute to abnormalities in attention to social stimuli in autism. Using ERPs, reactions were recorded in response to one standard and one deviant stimulus, generated for three stimulus classes ranging from acoustically simple to complex: simple tones, complex tones, and vowels. Nine high-functioning children with autism (mean age 8.9 yr) and 10 controls (mean age 8.4 yrs) were compared in sound detection by the sensory systems and in the transient encoding of acoustic sound features reflected by sensory ERPs. The results showed that sensory sound processing, including pitch discrimination, was intact in high-functioning children with autism, regardless of the acoustic sound complexity or ‘‘speechness’’. In contrast, their attentional orienting to sound changes was impaired only for speech sounds (the vowels). This finding demonstrates that high-functioning verbal children with autism, despite intact sensory processing of the spectral characteristics of sounds (ranging from simple tones, to complex tones, to vowels) are deficient in the involuntary orienting to changes in the vowel (speech sounds) but not to changes in simple or even complex tones (speechness sounds). If such an orientation deficit is present in infancy, it might severely compromise the development of verbal and non-verbal communication skills in this population.

In the same direction, Whitehouse and Bishop (2008) investigated whether children with autism are impaired in allocating attention to speech sounds or if they are characterized by sensory impairment in processing phonetic information. Event-related potentials of 15 children with high functioning autism and 15 TD controls were recorded in response to sounds. Participants heard two classes of stimuli: vowels and complex tones. An “oddball” paradigm was used, in which a sequence of repetitive identical sounds was presented with a “deviant” or a “novel” sound interposed. A distinctive brain response was measured, the mismatch negativity (MMN), which reflects the memory trace of the standard and the new stimuli. The findings indicate that children with autism have attenuated ERPs to speech but not complex tones. Both the perceptual and early cognitive processes of speech encoding were impaired in children with autism. In contrast, the speech and the non speech stimuli elicited highly similar ERPs waves from TD controls.

All these studies demonstrated that a lack of attention to speech, rather than an actual inability to perceive the physical characteristics of language, might be responsible for the pervasive and marked indifference to language and conversation observed in children with ASD. Indeed, deficits in social orienting, like failure to orient to one's name (see for example Dawson, Toth, Abbott, Osterling, Munson, Estes and Liaw, 2004) and a lack of joint attention (see for example Mundy, 1995) are well documented. Moreover, ASD children are often initially misdiagnosed as deaf (Rapin and Katzman, 1998).

On the basis of these considerations, Kuhl, Coffey-Corina, Padden, and Dawson (2005) investigated, for the first time together, the relationship between observed social orienting to speech and the processing of linguistic information in preschool children with autism, in the hypothesis that these two competences might be related. The social processing of speech was measured in an auditory preference task that involved "motherese" speech samples against non speech analogues of the same signals. Highly matched speech and non-speech signals were used in this study. The linguistic processing was measured in a phonetic discrimination task assessed with MMN, a measure of automatic, preconscious change detection. 29 children with ASD (mean age=45.31 months) were compared with 29 TD, chronologically matched and mental age matched, children. The results showed that children with ASD, differently from controls, demonstrated a preference for the non-speech analogue signals. Moreover, they failed to show a significant MMN in response to a syllable change. When ASD children were divided into subgroups based on auditory preference, and the ERP data reanalyzed, ASD children who preferred non-speech still failed to show an MMN, whereas ASD children who preferred motherese did not differ from the controls. These results support the hypothesis of an association between social and linguistic processing in children with ASD, leading to the conclusion that children with ASD who fail to orient to language might be impaired also in speech discrimination.

Deficits in auditory processing in individuals with ASD seem to be related not only to a lack of interest in speech or to a deficit in language processing, but also to an abnormally enhanced auditory perception of sounds. Jarvinen-Pasley, Wallace, Ramus, Happè, and Heaton (2008) investigated perceptual and semantic speech processing in verbally fluent children with autism and controls. A number of studies have proposed that a bias towards low-level perceptual information which may compromise higher-level language processing in such individuals (see for example Dakin and Frith, 2005; Happè and Frith, 2006; Simmons, Robertson, McKay, Toal, McAleer, and Pollick, 2009). Thus, this study investigated the existence of processing biases in children with autism and matched controls by the means of two experiments, which employed linguistic stimuli with competing low-level/perceptual and high-level/semantic information. The results showed that, whereas controls demonstrated a tendency to process speech semantically, children with autism exhibited superior perceptual processing of speech relative to controls, and showed no evidence of either a perceptual or semantic processing bias. These results seems to indicate the children with ASD tend to focus with equal attention toward simple or complex acoustic components of speech, losing important information about the semantic content of the verbal messages.

Heaton, Hudry, Ludlow, and Hill (2008) and Heaton, Williams, Cummins, and Happé (2008) investigated pitch contour discrimination in monosyllabic real words and monosyllabic nonsense words for 10 vowels sounds. Non-speech pitch stimuli were also presented. Children with ASD showed enhanced pitch discrimination across the three categories of sounds compared to controls. However, pitch discrimination was lower when the stimuli were speech like (both words and nonsense words), compared to non-speech stimuli. The use of non-words allows the conclusion that it was speech, rather than the semantic content, that could be the cause of poorer speech discrimination.

Jones, Happé Baird, Simonoff, Marsden, et al. (2009) further investigated this phenomenon in a large sample of individuals with ASD. 72 adolescents with ASD were compared with 57 age-matched controls in their ability to discriminate the frequency, intensity and duration differences in pairs of sounds. The results didn't show any difference between groups in the auditory discrimination ability. However, there was a subgroup of 20% of individuals in the ASD group who showed enhanced frequency discrimination skills and who were characterised by average intellectual ability and delayed language onset. This study in a large sample demonstrated that enhanced frequency discrimination is present in around 1 in 5 individuals with ASD and may represent a specific sub-phenotype, in which language onset is delayed. Individual differences in auditory discrimination ability in ASD presumably affects language comprehension, by modulating the degree to which sounds are detected or missed in the linguistic environment.

In summary, children with ASD seem to be characterized by a lack of attention to language, which is not grounded of difficulties in processing of the audiometric properties of pure or complex sounds, like pitch and frequency. On the contrary, enhanced sound perception seems to characterize this population compared to TD children, which in turn might interfere with their ability to consider the semantic meaning of the linguistic information. All these studies therefore indicate that it is not that children with ASD don't perceive language, it is that they don't attend to speech properly and therefore they tend to lose a quantity of relevant information in the course of daily conversation.

1.3.2 Auditory processing deficits in Asperger's syndrome

A lack of preference for speech has been found not only in individuals with autism but also in people with Asperger's syndrome. Asperger's syndrome is an Autism Spectrum Disorder characterized by impairment in social interaction and by restricted and repetitive patterns of behaviour and interests. It's different from the other disorders of the spectrum because of its relatively preserved linguistic and cognitive abilities. Therefore, it is reasonable to question whether auditory processing skills might be preserved in individuals with this specific disorder.

In line with these considerations, Alcàntara, Weisblatt, Moore, and Bolton (2004) analysed the difficulties in understanding speech in high-functioning individuals with autism or Asperger's syndrome. They aimed to evaluate the difficulties experienced in speech-in-noise perception in this population, testing a small group of high-functioning individuals with autism in comparison to age/IQ-matched normal-hearing controls. The results showed that the abilities of individuals with Asperger's syndrome were generally worse than controls, even controlling for different experimental conditions.

These results were confirmed by Lepistö, Silokallio, Nieminen-von Wendt, Alku, Näätänen, and Kujala (2006), who investigated whether the same deficits in auditory processing that characterize children with autism might also characterize individuals with Asperger's syndrome. To test this hypothesis a group of children with

Asperger's syndrome and their controls were tested for auditory processing using the ERP paradigm that was previously applied to children with autism. The results for the children with Asperger's syndrome were relatively similar to those previously obtained from children with autism using the same paradigm.

Very recently Kujala, Kuuluvainen, Saalasti, Jansson-Verkasalo, von Wendt, and Lepisto (2010) confirmed the lack of speech perception in Asperger's syndrome. This study aimed to determined speech feature discrimination in children with Asperger's syndrome in a multi-feature MMN paradigm, which resembles the complexity of daily conversation. This paradigm allows one to record cortical responses to five different speech-sound features in a constantly varying auditory environment, very similar to a natural speech environment. The results showed that children with Asperger's syndrome had larger cortical responses for intensity (hypersensitivity) and smaller cortical responses for frequency changes (hyphosensitivity) compared controls. The results of this study are of particular interest because, thanks to the characteristics of the stimuli used, highly similar to language, they might be reasonably generalized to the complexity of daily life conversations.

A further proof that auditory processing deficits characterizes ASD as a group of disorder derives from Kallstrand, Olsson, Nehlstedt, Skold, and Nielzen (2010), who wanted to verify whether the abnormal auditory information processing reported in individuals with Asperger's syndrome is a distinctive features of this population or if it is common with other clinical populations. To achieve this aim, individuals with Asperger's syndrome were compared to normally developing individuals, schizophrenic patients and attention deficit hyperactivity disorder (ADHD) patients. The results showed clear differences in auditory brainstem responses of individuals with Asperger's syndrome when compared to the other groups, indicating a specificity of such a deficit in Asperger's syndrome.

All these studies demonstrate that auditory deficits are distinctive to ASD, at different levels of language impairment ranging from autistic disorders, in which the social and linguistic deficit is macroscopic and long-lasting in most of the cases, to individuals with Asperger's syndrome, characterized by adequate or even advanced linguistic competences. Since such a deficit is not present in other neurological conditions, like schizophrenia or ADHD, it seems to be a rather distinctive feature that puts individuals in the autistic spectrum particularly at risk of not attending to voices and speech, despite different levels of linguistic abilities.

1.3.3 Neurological basis of auditory processing deficits in ASD

Due to the robust finding of a lack of auditory processing in individuals with ASD, a number of studies tried to understand the neurological basis of the deficiencies in auditory processing. Motivated to find a possible etiology of language abnormalities in individuals with ASD, mostly described by clinical evaluations in combination with atypical sensory sound processing, Gage, Siegel, Callen, and Roberts (2003) investigated frequency encoding mechanisms in auditory cortex in 15 children with autism (mean age=11.4 yrs) compared with 17 TD children of the same chronological age. As in previous work investigating frequency encoding mechanisms in auditory cortex in adults, this study provided evidence that the latency of the auditory evoked M100 is strongly proportional to frequency, with low frequency (100–200 Hz) tones associated with ~30 ms longer latencies than mid-range frequency (1–2 kHz) tones. Results indicate that for control children, the dynamic range of frequency modulation was similar to previous reports for healthy adults. Children with autism had a much reduced range of modulation in right hemisphere sites. These findings indicate that spectral decoding mechanisms in that hemisphere may be disrupted or impaired in autism, which might reflect a differential path in the maturation of frequency resolution mechanisms in auditory cortex in children with autism. Indeed, age-related changes in the latency of auditory evoked components (such as the M100, N1) have been related to maturational changes that occur during development such as myelination, synaptogenesis, dendritic pruning, and lamination of cortical layers (Ponton, Eggermont, Khosla, Kwong, and Don, 2002). As neural systems mature, conduction rates increase, decreasing the time to peak latency in evoked components. The slower neural conduction velocities in right hemisphere auditory fields in children with autism may therefore be a contributing factor to the auditory processing deficit found in this study.

Other studies indicate poor activation of left speech-related temporal areas in individuals with autism when listening to speech. Boddaert, Chabane, Belin, Bourgeois, Royer, et al. (2004) investigated whether a dysfunction of specific temporal regions specializing in the perception and integration of complex sounds, found in a previous study in adults with autism, was also present in children with autism. Synthetic non-verbal speech-like auditory stimuli were employed, characterized by speech-like formants that change over time. Their acoustic structure was similar to consonant-vowel-consonant sequence. However, normal volunteers never recognized them as speech. Eleven autistic children and six non-autistic mentally retarded children were assessed with positron emission tomography for regional cerebral blood flow in two conditions: during rest and while they were listening to speech-like sounds. The results showed, as in autistic adults, less activation in the left speech-related areas in autistic children compared to controls. In addition, a diffuse activation outside the temporal lobe was observed in autistic children, meaning that listening to complex sounds induces an abnormal cortical activation in this population. These results are of particular interest, because the abnormal cortical auditory processing observed in both children and adults with autism could be involved in inadequate behavioural responses to sounds and in language impairments characteristic of autism. This result might explain why autistic children often show exaggerated behavioural responses to sounds. Moreover, abnormal patterns of activation found in autistic adults could reflect basic anomalies in pre-linguistic auditory processing which might have a cascade effect on consequent language development. A reduced leftward asymmetry seems to be a robust finding, confirmed by a number of studies (see for example Boddaert, Belin, Chabane, Poline et al., 2003; Muller, Chugani, Behen, Rothermel, Muzik, et al., 1998; Muller, Behen, Rothermel, Chugani, Mizik et al., 1999; Redcay and Courchesne, 2008).

Another study considered cortical responses to speech in children with autism (Gomot, Belmonte, Bullmore, Bernard, and Baron-Cohen, 2008), examining neural activation patterns during auditory novelty detection in children with autism and controls. Results showed a more widespread network of brain activation associated with novelty detection in individuals with autism, which might be counterproductive during flexible social interactions. However, it is possible that some of the brain activity differences observed in this study may arise in part from comparison of two haemodynamic response functions that are out of synchrony with each other. For this reasons, these results need to be considered with caution and need replication.

Bruneau, Rogier, Malv, Bonnet-Brilhault, and Barthelemy (2010) investigated voice processing in both adults and children with autism in comparison to controls using cortical auditory evoked potentials. Surprisingly, a cortical response to voice was recorded in children with autism, which might reflect the activation of temporal voice areas typical in normally developing individuals. This response was not recorded at adult age in autism, leading to the interesting hypothesis that the lack of social interaction in children with autism might result in an "extinction" of the function of the temporal voice areas in adulthood. However, the study employed a small number of subjects so these results also need to be considered with caution.

All these studies demonstrate that autistic auditory behaviour, characterized by enhanced pitch processing abilities that often coexists with reduced orienting toward complex speech sounds, may result from atypical activity in auditory cortex. However, the cortex is not the only level of processing at which auditory processing seems to be impaired in ASD individuals.

Redcay (2008) illustrated how the superior temporal sulcus (STS), a brain region which is involved in language and social attention, plays an important role in analysing changing sequences of input (both in the auditory and visual domain), and interpreting the communicative significance of those inputs. Specifically, the STS separates the sequences of inputs into discrete units and extracts a meaning from these units. Most importantly, Redcay argued that because of the STS's role in interpreting social and speech input, impairments in STS function may underlie many of the social and language abnormalities seen in autism.

Empirical evidence of a reduced activation of the "voice area" in the STS has been reported in autistic adults by Gervais, Belin, Boddaert, Leboyer, Coez, et al. (2004). Further empirical evidence of a reduced activation of the STS in ASDs was presented by Samson, Hyde, Bertone, Soulières, Mendrek, et al. (2011). By employing fMRI to explore the neural basis of complex social sound processing, 15 autistic and 13 non autistic participants were tested with non-linguistic sounds which varied in spectral and temporal complexity. Subjects listened to the stimuli, indicated by pressing a button, if the sound was modulated or not. The results showed that the detection task was performed similarly by autistics and non autistics. The fMRI measures showed that, in both groups, increasing spectral complexity was associated with activity increasing in primary and non primary auditory cortex. Increasing temporal complexity was associated with greater activity in anterolateral STS in non autistics and a greater effect in primary acoustic cortex. Autistics exhibited diminished activity in the secondary auditory cortex and increased activity in the primary auditory cortex in response to the presentation of temporally but not spectrally complex sounds. Greater temporal complexity effects in regions sensitive to acoustic features and reduced temporal complexity effects in region sensitive to more abstract sound features could represent a greater focus toward perceptual aspects of speech sounds in autism.

Other authors investigated whether deficiencies in auditory processing which have been detected in both perception and cortical encoding of speech sounds might be the result of abnormal early processing and transcription of speech sounds, like for example at brainstem levels. Russo, Trent, Trommer, Zecker, and Kraus (2009) measured sub-cortical responses to syllables in children with and without autism. The aim of the study was to define "good" indices of auditory pathway function both in quiet and in challenging listening situations. The results showed that children with autism exhibited deficits in encoding timing and frequency of speech sounds but not in encoding non-speech sounds. Children with autism also exhibited a significant degradation of sub-cortical responses to speech presented in background noise in comparison to TD children. This study has a particular relevance in the field, because it provides solid empirical evidence of impairments in sub-cortical auditory processing in autism in relation to speech sounds. Abnormally low activity in the early part of the auditory brainstem response to complex sound stimuli was confirmed also by Kallstrand, Olsson, Nehlsted, Skold, and Nielzen (2010).

Moreover, other centers seem to be involved in atypical auditory processing in ASD, like the insula. Anderson, Lange, Froehlich, DuBray, Druzgal et al. (2010) evaluated receptive language processing in autism and controls using fMRI images. Areas of differential activation between groups were identified. Individuals with autism showed a significantly decreased activation in the left posterior insula compared to controls. These results are consisted with previous findings showing impaired emotive processing of language in autism, which in turn might play an important role in language development. However, this study was limited because the linguistic stimuli employed lacked of contextual properties and therefore they were significantly different from the speech used in natural conversations.

In summary, auditory processing of speech seems to be compromised in adults and children with ASD at different levels of processing. Abnormal responsiveness ranging from the auditory cortex to the STS, the brainstem and the insula have been found. Such abnormalities, if present in infancy, might account for abnormal social orienting and reduced processing of speech in ASD.

1.3.4 Theoretical explanations of auditory processing deficits in ASD

Some authors tried to explain the auditory processing abnormalities of individuals with ASD in the light of specific conceptual frameworks. Siegal and Blades (2003) discussed auditory processing deficits in the light of the debate over the role of the Theory of Mind framework in explaining the social deficit in ASD.

They proposed an interesting connection between autistic symptoms and auditory processing disruptions, in which the auditory processing deficit in ASD might be considered as possible precursor of Theory of Mind impairments in this population. The starting point for this proposal was that the absence of 'Theory of Mind' (ToM) reasoning (which consists in the ability to attribute mental states to others, like beliefs or thoughts) often characterizes individuals with ASD (see for example Siegal and Varley, 2002).

An essential ability in the recognition that the minds of others contain mental states, which might correspond but also differ from reality, is auditory processing. It is thanks to this ability that children extract the linguistic information received through auditory perception, necessary to participate in conversation and social interactions with others and to learn about the mind, the real word and their reciprocal relationships.

According to Siegal and Blades (2003), several studies demonstrate that children with ASD have deficits in auditory processing that preclude participation in conversation with others, which possibly leads to downstream impairments on ToM tasks, and excessive interest in objects at the expense of people. Moreover, there is much evidence that children whose language is severely impaired, especially relative to their non-verbal intelligence, are at risk of developing severe symptoms of autism. Therefore, auditory processing skills might be pivotal abilities for ToM to be developed, and hence needs to be further investigated from that perspective.

Another theoretical account that has been used to explain auditory deficits in ASD is the Weak Central Coherence Theory (WCC). The WCC attempts to explain ASD patterns of information processing in all modalities (see for example Frith, 2003). According to this theory, adequate information processing requires the integration of local elements into a ‘Gestalt’. Such an information processing strategy is referred to as ‘central coherence’. The WCC theory postulates that individuals with ASD show a deficiency in this central coherence tendency, thus in their ability to process ‘global’ information. This deficiency is thought to result from a bias towards processing ‘local’, detailed information, which is expected to be enhanced.

Foxton, Talcott, Witton, Brace, McIntyre, and Griffiths (2003) demonstrated empirically that the WCC known in ASD in the visual domain might be extended to sound patterns in this population. A set of auditory tests were administered to a small sample of children with autism and their controls. The results demonstrated a “global” bias in sound perception in controls but not in individuals with autism, leading to the conclusion that individuals with autism are not susceptible to interference from an auditory `coherent gestalt'. However, the large pitch differences of the stimuli used might have interfered with these results so replications are needed to confirm a deficit in WCC in ASDs in auditory perception.

Haesen, Boets, and Wagemans (2010) reviewed the most significant behavioural and electrophysiological studies on auditory processing in autism in the light of WCC, organized according to the methodology used in the studies (behavioural versus electrophysiological measures) and to the stimulus complexity (simple versus complex sounds). The aim was to verify whether individuals with autism show a locally oriented processing style in auditory perception, in line with the WCC of autism. The findings revealed an intact or even enhanced local auditory perception in individuals with autism, while a poorer processing of global auditory features needs further empirical confirmation.

A third theoretical account which has been used to explain auditory deficits in ASD is the ‘‘Enhanced Perceptual Functioning” (EPF) model. This model, which arises out of years of empirical findings, defines autistic visual perception as characterized by a locally oriented and enhanced low-level perceptual functioning (Mottron and Burack, 2001). A number of empirical studies reported a reduced performance in the processing of static and dynamic second-order stimuli, and a superior performance in the discrimination of first-order stimuli in ASD (Bertone and Faubert, 2003; Bertone, Mottron, Jelenic, and Faubert, 2005). First-order information are considered to be ‘‘simple’’, while second order information is considered to be ‘‘complex’’ because it recruits more extensive neural circuitry as well as additional processing prior to orientation identification. Based on these empirical evidences, Bertone et al. (2005) concluded that enhanced sensitivity for first-order information and reduced sensitivity for second-order information detection in autism characterize their atypical visual processing.

Bonnel, McAdams, Smith, Berthiaume, Bertone, et al. (2010) investigated whether, on the basis of some structural and functional parallels between visual and auditory perception, the framework of the EPF model (Mottron, Dawson, Soulie`res, Hubert, and Burack, 2006) could be extended to explain the abnormal auditory perception for speech in individuals with autism. The participants of Bonnel et al. study (2010) were adolescents and young adults with autism, Asperger's syndrome, and typical developmental histories, all with IQs in the normal range. Consistent with the EPF model of ASD (Mottron et al., 2006), the participants with autism, but not with Asperger's syndrome, displayed enhanced pitch discrimination for simple tones. However, no discrimination-threshold differences were found between the participants with ASD and the TD controls across spectrally and temporally complex conditions. These findings indicate that enhanced pure-tone pitch discrimination may be a cognitive correlate of speech-delay among persons with ASD. However, auditory discrimination among this group does not appear to be directly contingent on the spectro-temporal complexity of the stimuli. The hypothesis of an association between enhanced perceptual abilities and delayed speech onset among persons with autism is further supported by Mottron, Soulieres, Meilleur, and Dawson (2008), who highlighted a strong association between visuo-motor peaks of abilities, as evident in enhanced performance in the block design subtest of the Wechsler Intelligence Scales, and a history of delayed onset of first words and sentences among persons with autism. These patterns of findings suggest that visual and auditory perceptual peaks of ability may constitute cognitive correlates of delayed speech onset among autistics as well as a phenotypic marker of the distinction between autism and Asperger's syndrome.

In summary, the auditory processing deficit in ASD is a robust finding that has been extensively studied. This deficit is present not only in autistic individuals but in all the spectrum, even in Asperger's patients, who are known to not be delayed in language development. Moreover, it is a distinctive deficit for ASD, because it is not present in other syndromes. This deficit is not grounded in abnormalities in acoustic perception of sounds but it seems specific to voices and speech. Thanks to the new advances in electrophysiological and neuroimaging techniques, brain responses to speech have been investigated in ASD. Abnormal processing at different levels, ranging from the auditory cortex to the STS, the insula and the brainstem have been found. These abnormalities, if present in infancy, might account for abnormal social orienting and reduced processing of speech in ASD. Even though a unique theoretical account of auditory processing deficit in ASD has not been found, there have been some attempts to explain these abnormalities in terms of ToM, WCC and EPM. Each of these models contributes to some extent to define the puzzling enigma of auditory processing deficits in ASD.

1.4 The roots of contamination sensitivity: intuitive perception of disgust

Classical contamination sensitivity tasks (for example Siegal and Share, 1990) evaluate behavioural reactions to contaminant by asking children if it's OK to drink a juice that has been contaminated by an insect. These tasks are meant to be behavioural, in which the behaviour of the child tells us something about his/her understanding of contamination sensitivity: if a child refuses to drink the juice, it means that he\she understands the contaminating nature of the insect. However, this kind of task evaluates an explicit understanding of contamination sensitivity, which implies a number of cognitive and linguistic abilities. In order to be successful in this task, a child needs to communicate his/her choice to the experimenter, either verbally or non-verbally. Secondly, the child needs to inhibit his/her impulse to drink a liked drink (the juice) because he/she knows that the juice has been contaminated. Such inhibition requires a quite intact executive control, which is known to be compromised in children with ASD (Hill, 2004). These children are also characterized by difficulties in language, communication and social interaction (Tager-Flusberg, 1996) which might mask, in combination with a lack of inhibition, the actual knowledge about contamination in these children as measured by behavioural tasks.

For these reasons, nonverbal tasks might provide a more sensitive measure of contamination sensitivity, because they don't require either a verbal judgement or a voluntary choice between two possible alternatives. With this considerations in mind, for the purpose of our studies we decided to use two measures in combination that might correct respectively for socio-communication and executive functioning deficits in children with ASD: preferential looking and variation in pupil size.

Preferential looking measures will be obtained by tracking visual attention as an index of “preference” toward one of two identical glasses of juice: one glass that has been contaminated with an insect and another glass containing uncontaminated juice. By measuring the length of the observation toward the two glasses, we could infer whether the children discriminate between the contaminated vs the uncontaminated juice. If the children look longer on average at the uncontaminated juice, we would assume that they “*know*” the difference between the two glasses, which appear identical but that are different because one has been contaminated by an insect.

Variations in pupil size are an involuntary, unconscious response to contaminants, controlled by sub-cortical components of the nervous system (like the locus coeruleus). Thus, this phenomenon is completely independent by the frontal cortex and therefore it won't be affected by any possible deficits in executive functioning. This measure will be obtaining by comparing the pupil size before and after stimuli onset, by controlling for luminosity and cognitive load in the visual stimuli presented. The two measures will be recorded by the means of an eye-tracker, which has been widely used in the last years to access visual attention in ASD.

1.4.2 Eye tracking studies in ASD

Eye tracking technology has been widely used to explore how children process visual stimuli during task completion. The assumption behind tracking eye movements is that when an individual fixates an object its image falls on the fovea, the part of the retina specialized for detailed visual processing. For this reason, recording gaze behaviour can indicate where a person is seeking information from when exploring a visual scene, which in turn highlights the strategies that the individual is using to complete the task. Eye-tracking allows an objective and quantitative observation of visual behaviour, indicating which information from a scene is available to the brain moment by moment (Boraston and Blakemore, 2007).

Eye-tracking has been successfully used with children with ASD in a variety of studies. The majority of the eye-tracking studies in ASD investigated gaze patterns in observing social stimuli. These studies typically involved images or video clips of human faces or people, either performing some action or engaged in social interactions.

The most striking and robust finding of these studies is that, while TD adults fixate mainly on the eyes but also on the nose and mouth when exploring a scene involving other individuals (see for example the classical study of Luria & Strauss, 1978), individuals with autism tend to ignore these so called “core features” and tend to focus mainly on socially irrelevant parts of the stimuli (see for example Klin, Jones, Schultz, Volkmar & Cohen, 2002; Phelphrey, Sasson, Reznick, Paul, Goldman and Piven, 2002; Riby, Doherty-Sneddon, and Bruce, 2009). However, other studies of individuals with autism found no differences in gaze patterns between autistic participants and controls (van der Geest and Frens, 2002; Freeth, Ropar, Chapman, and Mitchell, 2010; Freeth, Chapman, Ropar, and Mitchell, 2010).

These differing results highlight the importance of considering a number of possible intervening factors in interpreting the results of eye-tracking studies. First, the characteristics of the participant groups can make a difference, whether they are adults, children, or individuals trained in the recognition of specific stimuli. Most importantly, it is critical to consider the nature of the stimuli used. It has been suggested that gaze differences between autistic individuals and controls only exist in response to dynamic stimuli such as video clips (e.g. Klin et al. 2002; Pelphrey et al. 2002; Dalton, Nacewicz, Johnstone, Schaefer, Gernsbacker, et al. 2005). Studies that failed to find a difference have used static stimuli (e.g. van der Geest et al. 2002). Of course video clips are more complex compared to static images, but they are also more ecologically valid in that they simulate a real-life social situation characterized by a variety of people and objects in the scene. The use of one kind of stimuli or another should be carefully considered in relation to the specific research questions of each study.

Moreover, fixation patterns unfortunately not directly indicate how the brain uses the visual information it receives. For example, even if an individual shows normal fixation of the eyes, he or she may not make use of the information available in the eyes. Therefore, to explore which specific information is used to solve a task, specific experimental paradigms need to be used in combination with eye tracking technology. For example, Spezio, Po-Yim, Castelli, and Adolph (2007) used eye-tracking together with a novel method of presenting stimuli, the “Bubbles” method (Gosselin and Schyns, 2001), to investigate which parts of the face subjects were using to recognize emotional expressions. The “Bubbles” method allows to create pictures in which only certain parts of the face are visible, by combining static facial stimuli with an algorithm developed to vary the facial information available on any given trial.

Each trial shows only randomly revealed parts of the face, determined by the number of “bubbles,” or Gaussian holes in a mask (called the “Bubbles” mask) covering the underlying image (a static facial stimuli). Thus, each trial in the “Bubbles” paradigm reveals to the participant some areas of the face while obscuring others. The more bubbles there are, the greater the portion of the face that is revealed to a viewer. Spezio et al. (2007) used the "Bubbles" method to vary the facial information available during an emotion recognition task, and measured the eye movements made as participants viewed these stimuli. The results confirmed that individuals with ASD had a greater reliance on information from the mouth in order to identify an emotion. Also, the specific instructions given to the participant could significantly impact performance of individuals with ASD in eye tracking paradigms. There might be differences in the gaze strategies used when a subject is completing a specific task versus differences in spontaneous behaviour. Individuals with autism might conceivably look at the face in a normal way when required to do so by a task, yet fail to explore a face visually without specific reason to do so. On the other hand, they might show normal spontaneous gaze behaviour, but an inability to examine the appropriate parts of a face when performing the task (Boraston and Blakemore, 2008). In this regard, one study included both a free-viewing and a task-directed condition and found the same pattern of results in both (Pelphrey et al. 2002). However, different instructions need to be considering carefully when interpreting the results. In summary, this brief review of the potential use of eye-tracking technology in individuals with ASD is designed to convince the reader that the ideal task for investigating a specific aspect of function will vary according to the particular theoretical question under investigation. The validity of the results strongly depends upon the nature of the stimuli and the protocol used in each specific study. Even though the non-intrusive nature of the eye-tracking technique and the use of videos of realistic social interactions can guarantee ecological validity to some extent, relevant critical limitations remain. The interpretation of the results needs always to consider that eye tracking studies usually do not involve real people, but mainly static images or videos in which the behaviour of the protagonists is not contingent on the behaviour of the participants, as with a real person. Lastly, the majority of the studies with eye-tracking technology focus on the investigation of gaze patterns in relation to social relevant stimuli, like the faces and the eyes. It might be of interest to investigate whether individuals with ASD show abnormalities not only in fixating social relevant stimuli but also in the visual exploration of “cultural” relevant stimuli, like for example contaminants and disgust elicitors.

1.4.1 Pupillometry as a possible physiological measure of emotions and attention

An implicit understanding of contamination might be grounded in the unconscious perception of contaminants that precede a semantic interpretation of the stimuli. A number of studies demonstrate that people’s emotional reactions are triggered by what individuals unconsciously perceive (Prinz, 2004; Ruys). Following this “unaware” perception, specific details of the event are semantically interpreted and elicit specific emotional responses such as fear or disgust (Bargh & Chartrand, 2000).

Unfortunately, there are not data available so far on the realm of disgust. The phenomenon of the “unaware perception” of disgust might be particularly of interest for the study of contamination sensitivity in children with ASD, since it might be possible that children with ASD who don’t show contamination sensitivity might lack of an implicit understanding of the disgusting valence of the elicitors. A possible way to investigate implicit reaction to contaminant is the measurement of changes in pupil diameter, called “pupillometry”.

Pupil size changes in response to changes in ambient light. In darkness, the pupil can enlarge to an average size of about 7 mm with a standard deviation of 0.9 mm (MacLachlan and Howland, 2002). In standard light conditions, its average size is about 3 mm (Wyatt, 1995). Thus, changes in illumination can provoke pupillary dilations of more than double (about 120%) of its typical size. Pupil size also changes in response to emotionally relevant stimuli (Aboyoun and Dabbs, 1998; Hess and Polt, 1960; Hess, Seltzer, and Shlien, 1965) and in relation to specific cognitive mechanisms: increasing load in memory (Beatty and Kahneman, 1966; Chatham, Frank, and Munakata, 2009; Piquado, Isaacowitz, and Wingfield, 2010); difficulty of mental calculations (Ahern and Beatty, 1979); interference or competition between stimuli (Laeng, Orbo, Holmlund, and Miozzo, 2011; Moresi et al., 2008). Changes that are cognitively driven are rarely greater than 0.5 mm (Beatty and Lucero-Wagoner, 2000). The maximal dilation that can be elicited by psychologically relevant stimuli that are invariant in luminance is equal to a 20% of change (Hess and Polt, 1960).

While these results are quite robust and consistent, the relationship between affective processing and pupil size variation is still controversial. Hess (1972) suggested that there is a continuum ranging from extreme dilation to interesting or pleasing stimuli to extreme constriction to unpleasant or distasteful stimuli. In contrast, Janisse (1974) argued that there is no pupil constriction to negative stimuli. The constriction response, if present, may be limited to few individuals and to a small range of stimuli. Loewenfeld (1993) also studied the effects of various sensory and psychological stimuli to pupil size variation, finding that none of them caused pupil constriction except increased light intensities. In summary, the results of classical studies on pupil variation as a possible measure of emotional reaction are not straightforward. However, it is important to consider that the majority of these studies were done during the late 60s and the 70s. Then, pupillometry was abandoned for a while until recently, when new technology (like for example the eye-tracker) lead to a newest wave of interest in variations in pupil size as an index of implicit sensitivity to emotionally relevant stimuli.

Recent studies demonstrated that subliminal reward cues can trigger pupillary dilations that are proportional to the cues' value and to the level of demands in a cost–benefits in decision-making tasks (Bijleveld, Custers, & Aarts, 2009). Laeng and Falkenberg (2007) demonstrated that women dilated their pupils when watching looking at photographs of their boyfriends during the ovulatory stage of their cycle.

Weiskrantz, Cowey, and Barbur (1999) demonstrated that pupillary responses highlight residual visual capacity in neurological patients, beyond patients awareness. Weiskrantz, Cowey, & Le Mare (1998) found a reliable constriction of the pupil to visual stimuli presented within the blind field in patients who suffered localized brain damage to the visual cortex.

Taken together, these studies demonstrate that pupillary reactions might be considered as a reliable involuntary index of emotional perception in individuals at a pre-semantic level.

Very recently, neuroscientists identified a strong link between pupillary responses and the activation of the locus coeruleus and noradrenergic system. One current hypothesis is that the noradrenergic system, which originates in the locus coeruleus, mediates the functional integration of the brain's attentional system (Corbetta, Patel, & Shulman, 2008; Coull, Büchel, Friston, & Frith, 1999; Sara, 2009). Pupillary responses occur spontaneously as the result of neural inhibitory mechanism in the parasympathetic oculomotor complex by the locus coeruleus (Wilhelm, Wilhelm, & Ludtke, 1999). Robust findings have established that changes in pupil diameter are tightly correlated to changes in the activity of neurons in the locus coeruleus (Rajkowski, Kubiak, & Aston-Jones, 1993; Rajkowski, Majczynski, Clayton, & Aston-Jones, 2004).

The role of the subcortical structures linked with pupil dilation, like the amygdala and locus coeruleus, is to alert frontal cortical areas to give relevance to the new stimuli perceived (Duncan & Barrett, 2007; Gompf et al., 2010; Laeng et al., 2011; Liddell, Brown, Kemp, Barton, Das, et al., 2005; Sterpenich, D'Argembeay, Desseiles, Balteau, Alboy, et al., 2006). Thus, variations in pupil size indicate the presence of processing that takes place at a preconscious level, necessary for phenomenal awareness (Block, 2005; Chapman, Oka, Bradshaw, Jacobson, and Donaldson, 1999). In the light of this, pupillometry might be considered as a window into attention, providing an observable signal of the moment when an event becomes relevant before it consolidates into awareness.

Because pupillary response might be obtained even without participant knowledge (Bijleveld et al., 2009; Laeng and Falkenberg, 2007; Laeng, Sirois and Gredebäck, 2012), one potential application of pupillometry could involve subjects that cannot normally understand instructions or provide controlled, verbal responses, like for example infants (Gredebäck and Melinder, 2011; Jackson & Sirois, 2009) or individuals with neurological conditions which affects language and communication, like for example ASD. For the above reasons, we decided in to include pupillometry as a tool to evaluate intuitive, implicit knowledge about contamination in children with ASD.

1.5 Open questions and overview of the research in this Thesis

Children's understanding of biology and health has been extensively studied (see for example Siegal and Peterson, 1999). A number of cognitive abilities are thought to be responsible for this knowledge, like the distinction between appearance and reality (Siegal and Share, 1990), the concept of tiny, invisible particles that continue to exist in food even though the contaminant has been removed (Toyama, 1999; Au et al., 1993), the distinction between bodily and mental reactions to contamination (Kalish, 1997) and the understanding of the nature of germs and contagion (Raman & Gelman, 2005).

However, besides these cognitive grounds, contamination sensitivity seems to emerge through a process of basic socialization from parents and adults care-takers, who spend a considerable amount of time during the first two years of life trying to teach to the children what not to put into their mouths (Dunn, 1986; Toyama, 2000). A number of studies show a strong resemblance between children and parent’s preferences about food (Rozin et al., 1984; Stevenson et al., 2010). Moreover, a cross-cultural study demonstrated significant differences between Indian and American children's responses to situations of potential contamination (Hejmadi, Rozin, & Siegal, 2004). Despite the centrality of language for the development of children's biological knowledge about contamination, little is known about children who are impoverished in language, like children with ASD. Only one study so far has looked at contamination sensitivity in this population (Kalyva et al., 2009) and further work is clearly necessary.

The study of contamination sensitivity in children with ASD is of particular interest due to the protracted debate over the role of GI symptoms in the etiology of ASD. Contamination sensitivity might play a major role in preventing GI symptoms in these children, by promoting proper diet and hygiene. However, no studies, to the best of our knowledge, have ever investigated biological knowledge about disgust and contamination in these children in relation to GI problems.

Since disgust and contamination sensitivity are acquired through out a process of enculturalization, linguistic and communication deficits put children with ASD at risk of not learning about disgust and contamination in the course of the daily conversation with parents and care-givers. Language and communication impairments in these children are known to be grounded in auditory processing deficits, which lead to an abnormal perception of voices and speech at different levels, from the cortex to the brainstem. Despite the centrality of auditory processing in perceiving and focusing on language and communication, no studies so far have investigated the possible role of auditory processing in the development of biological concepts about disgust and contamination.

Another important issue in the study of contamination sensitivity in children with language and communication impairments, like children with ASD, is the validity of classical contamination sensitivity tasks (see for example Siegal and Share, 1990). These tasks evaluate behavioural reactions to contaminants by asking children if it's OK to drink a juice that has been in contact with and insect. In order to be successful in these tasks, the child needs to communicate with the experimenter, either verbally or non verbally. The difficulties in language and communication that characterize children with ASD might therefore render behavioural tasks particularly challenging for these children, and put them at risk of failing even though they possess a sense of contamination. Besides language and communicative difficulties, there are also possible deficits in executive functioning in individuals with ASD (Hill, 2004). In an experiment that offers a glass of a contaminated juice, children with ASD might be unable to refrain from drink due to a lack of inhibitory control, even though they know that the juice is not drinkable. Therefore, non-verbal tasks to access contamination sensitivity in children with ASD need to be developed.

Finally, all the studies investigating the role of enculturation in contamination sensitivity never considered the influence of explicit linguistic information about disgust and contamination on food rejection behaviour in children. The best way to verify whether language might exert some effect on contamination sensitivity is to say to the children that something is disgusting (like an insect) and to observe children's behaviour toward the food that has been contaminated after receiving this information. No studies so far have investigated how language might affect contamination sensitivity in such a direct way.

On the basis of these open questions in literature, my thesis aimed to answer to the following research questions:

1) are children with ASD, who are impoverished in language and communication, at a major risk to develop a reduced sense of contamination compared to typically developing children?
2) is a lack of contamination sensitivity in children with ASD related to GI problems?
3) if there is a subgroup of children with ASD that lack contamination sensitivity, would it be significantly lower in auditory processing skills compared to the children (ASD and TD) with contamination sensitivity?
4) is an explicit reaction to a contaminant based on an implicit sense of contamination?
5) does a linguistic information about contamination (like telling children that an insect is disgusting) affect implicit contamination sensitivity in ASD?

To answer to these questions, we developed four studies, which addressed respectively the first, the third, the fourth and the fifth research question listed before. The second question, concerning contamination sensitivity and GI problems, have been addressed in the second, the third and the fourth experiment. Study 1, described in Chapter 2, replicated Kalyva's et al. study (2009) in an attempt to estimate if and to what extent children with ASD lack contamination sensitivity, in comparison to TD controls and children with Learning Disabilities. Study 2, described in Chapter 3, evaluated contamination sensitivity in relation to auditory processing skills in children with ASD, in comparison to TD controls. Study 3, described in Chapter 4, evaluated implicit contamination sensitivity with a non-verbal paradigm, using eye tracking technology in ASD children and TD controls. Preferential looking was considered as non verbal index of contamination sensitivity, to avoid issues related with language and communication with the experimenter. Variations in pupil size were considered as involuntary reactions to contaminants, which might be an index of knowledge about disgust elicitors beyond deficits in executive functioning, which might affect gaze movements when exploring a visual scene. Study 4, described in Chapter 5, investigated the influence of explicit linguistic information about contaminants in a non-verbal contamination task both in children with ASD and TD controls. The children were told that there was an insect in the juice and that the insect is disgusting and their reactions in terms of preferential looking and variations in pupil size were analysed.

Finally, a synthesis of the results of the four studies (study 2, 3 and 4) concerning GI problems and contamination sensitivity is presented in the Chapter 6 of the Thesis.

2.1 Summary of the Chapter

This Study investigates how access to language might affect contamination sensitivity by comparing food rejection behaviour in TD preschool children, children with ASD (who are known to be impaired in language and social communication) and children with Down Syndrome (DS). The results showed that many children with ASD were ready to drink liquids that had been contaminated by insects. In contrast, the majority of TD children and children with DS demonstrated strong contamination sensitivity. This study replicated Kalyva et al. findings (2009), in which a lack of contamination sensitivity was found in children with ASD, in sharp contrast with TD and DS, for the first time. Our study also extended Kalyva et al. findings (2009), by employing three groups of children from the same nationality (Italy) and by considering the possible role of the concept of "invisible particles" in contamination sensitivity. Neither knowledge of the physical concept nor mental and non-mental representation skills accounted for differences in contamination sensitivity in children with ASD. Thus, the results point to the prominent role of access to language and communication in promoting contamination sensitivity.

2.2 Introduction

Contamination is a specific property of disgust elicitors, which might be conceptualized as the power of elicitors to convert an edible food into an inedible one by physical contact (Rozin et al., 1986). Cross-cultural core elicitors are generally decayed food and body products, ones for which the brain produces rejection behaviours in order to avoid biological pathogens (Rozin and Fallon, 1987; Rozin, Haidt, Fincher, 2009). Contamination sensitivity is present in adults (Fallon, Rozin, Pliner, 1984) and it develops in children at early age, during the preschool years (Au et al., 1993; Siegal and Share, 1990; Kalish et al., 1997; Legare, Wellman & Gelman, 2009).

Typically developing children are known to be naturally attuned to others' social and communicative signals, such us emotional expressions, since the first year of life, in a constant process of social referencing and social learning in which they are engaged during the course of natural conversation (Senju & Csibra, 2008). It is through engagement in conversations with others that children in Western countries and in Japan are instructed about food and contamination so that they learn about the edible-inedible distinction (Rozin, 1990; Siegal, 2008; Toyama, 2000).

Some studies show a level of parent-child resemblance in disgust sensitivity (Davey, Forster, Mayhew, 1993; Rozin, Fallon, Mandell, 1994; Stevenson et al., 2010). However, these are correlational studies, so this socio-cognitive hypothesis of contamination sensitivity development is still controversial and it needs to be further investigated. One possible way to assess how access to language and to family conversation affects the development of contamination sensitivity might be to observe food rejection behaviours in children with ASD.

While typically developing children develop their sensitivity to food contamination within their social context, children with ASD, who are known to be impaired in attending to voices, speech, and other social signals like gaze direction and joint attention (Ceponiene et al., 2003; Dawson et al., 2004; Kuhl, Coffey-Corina, Padden, & Dawson, 2005; Nadig et al., 2007), might be at risk of not developing an understanding of emotions like disgust. In fact, children with ASD may not be attentive to conversations about food initiated by caregivers who point out that substances which appear edible or drinkable may in reality be contaminated. In addition, reasoning about food contamination requires intact Theory of Mind competences, such as the ability to represent the intentions of the food server and an understanding that food that appears good may not be in reality, which are typically impaired in children with autism (Siegal and Blades, 2003).

On the basis of these considerations, Kalyva et al. (2009) investigated contamination sensitivity in TD pre-school children, children ASD and DS children. A classical contamination task was employed (Siegal and Share, 1990), in which the experimenter, in the course of natural conversation during their snack time, told the children ‘‘Here’s some juice. Oh! It has a cockroach in it!”. The cockroach floated on top of the juice. The experimenter removed it from sight without a trace and asked, ‘‘Is the juice OK or not OK to drink?’’. Children with contamination sensitivity should refuse to drink the juice. In addition, as reasoning about food requires representation of intentions of the food server and an understanding that food which was good may become inedible after a transformation, hence the children were given Theory of Mind measures of mental representation and false photograph measures of non-mental representation.

The results of Kalyva's study demonstrated that, while the majority of TD and DS showed contamination sensitivity, many children with ASD were prepared to drink liquids that had been contaminated by insects. The children were equivalent to the others in verbal and non-verbal mental age, Theory of Mind abilities and non-mental representation. There was evidence for a developmental delay as contamination sensitivity in autism was associated with increasing age.

However, this is the only study to date and hence this important result needs replicating. Moreover, in this study, the ASD children and some of the TD controls were Italian, while the DS children and some of the TD controls were from Greece. If the process of enculturation is a key component in the development of contamination sensitivity, it is important to replicate the study controlling for this possible intervene variable.

Our study aimed to replicate Kalyva's study, by investigating food rejection behaviour in ASD in comparison to TD and DS children. Differently from Kalyva et al. study, children of the same nationality were employed (Italy). Measures of verbal and non-verbal mental age in ASD children and in children with DS were considered. As in Kalyva et al. study (2009), the children were given Theory of Mind measures of mental representation and false photograph measures of non-mental representation. In addition, a measure of the concept that tiny, invisible particles continue to exist in the juice even though the contaminant has been removed was employed.

We hypothesized that: 1) children with ASD will lack contamination sensitivity in comparison to the other two groups; 2) children with ASD who lack contamination sensitivity might be lower in the ability to reason about invisible particles in the liquids.

2.3 METHOD

Participants

The participants in the study were 28 TD children (13 M; 15 F), aged between 4 and 5 years (*M* = 5 years, *SD*=7), and 20 children with ASD (15 M; 5 F), aged between 4 and 14 years (*M* = 8.2 years, *SD*=29), diagnosed by expert clinicians on the basis of the DSM-IV criteria and the ADOS (Lord et al., 1999), and 10 children with Down syndrome (DS; 6M; 4F), aged between 4 and 14 years (*M* = 10.9 years, *SD*=29). Children with DS had a non verbal mental ages between 4 and 5 years. Both children with ASD and with learning disabilities were delayed in development and therefore they were older in chronological age compared to TD children. The children were recruited in public schools in Cagliari (Italy) while ASD children were recruited through referrals in a Centre for Pervasive Developmental Disorders in Cagliari (Italy). Children with DS were recruited through referrals in different Neuropsychiatry Units in Sardinia (Italy).

Procedure

All the children were tested individually, in a quiet room, with written parental consent. The TD children were tested at school, after a period of one week of familiarization, in which the experimenter collaborated with the teachers during classroom activities. The familiarization is meant to avoid any possible distress that an interaction with an unfamiliar adult might cause to the young children. The testing was organized according to the children's need to follow the regular activities of the school, in strictly collaboration with the teachers. The children with ASD were tested at the Centre for Pervasive Developmental Disorders at the Hospital "G.Brotzu" in Cagliari or at school, while the children with DS were tested in different Neuropsychiatry Units in Sardinia (Italy). Again, the testing occurred after a period of familiarization with the participants. The experimenter collaborated with the therapists and the psychologist in activities involving the child at the Hospital, for one week before testing. The testing was organized according to the children's need to follow the regular activities at the Hospital, in strictly collaboration with parents, doctors, therapists and psychologist involved in the children's program.

Measures

All the children in the three groups were tested for contamination sensitivity with a measure used by Siegal & Share (1990). In the course of natural conversation during their snack time, the experimenter told to the children: "Here's some juice. Oh! It has a cockroach floating on top" (Fig. 2.1). The experimenter removed the insect without a trace and asked, "Is the juice OK or not OK to drink?".

Figure 2.1: A glass of juice contaminated by an insect used in the contamination sensitivity task.

She continued by telling the children a story in which the task will be to evaluate another's responses to contamination:

> *"A grown-up poured juice into a glass with a cockroach in it. The cockroach floated on top and then the grown-up threw it away. He asked Luca (Marta, for the girls), a boy (girl) your age, whether the juice would be Ok to drink or whether it would make him (her) sick. Luca (Marta) would say that the juice would be OK to drink. (Test question:) Was Luca (Marta) right or would it make him/her sick?"*

The order of presentation of the two alternatives in the test question ("*Was Luca (Marta) right or would it make him/her sick?"*) was counterbalanced across children, in order to avoid a possible bias in children's responses, due to a fixed last alternative. Children with ASD were tested for non verbal mental abilities with the Leiter International Performance Scale-Revised (Roid and Miller, 1997). Children with Down syndrome were tested with the Wechsler Intelligence Scales (Wechsler, 1991). The children with ASD and the children with DS were tested for verbal mental age with the Italian version of the Peabody Picture Vocabulary Test (Dunn & Dunn, 2000). Children with ASD were also given a test of false beliefs (Woolfe, Want, & Siegal, 2002), a test of non-mental representation (Zaitchik, 1990) and a test of conservation of invisible particles after dissolution (Rosen and Rozin, 1993).

The False Belief Task (Woolfe, Want, & Siegal, 2002).

The children with autism received a test of false beliefs (Woolfe, Want & Siegal, 2002) used in previous studies with deaf children (Meristo, Falkman, Hjelmquist, Tedoldi, Surian et al., 2007). They were shown four ‘‘thought pictures’’, adapted from a procedure used by Custer (1996): two in a false belief condition (FB – see an example in Fig. 2.2), and the other two in a true belief condition (TB – see an example in Fig. 2.3). The four thought pictures were: (a) a boy fishing thinks he has caught a fish (TB=fish/FB=boot); (b) a girl thinks she sees a tall boy over a fence (TB=a tall boy/FB=a small boy standing on a box); (c) a man thinks he is reaching into a cupboard for a drink (TB=a drink/ FB=a mouse); and (d) a man thinks he sees a fish in the sea (TB=a fish/FB=a mermaid). The content of the items of FB and TB tasks was randomised across children. For each task, children were asked a belief and a reality question. They were scored as having passed the task if they answered both questions correctly. Each child therefore received a FB and a TB score ranging from 0 to 4.

Figure 2.2: An example of the Theory of Mind task used in this study, reprinted by permission from MacMillan Publishers Ltd: Siegal, M., Varley, R. (2002). Neural systems involved in “Theory of Mind”, *Nature Neuroscience*, Volume 3, pp. 463-471. Children were shown a picture of a boy fishing, and in this example the boy has caught a boot. The children were then shown four pictures (a fish, a boot, and two distracter objects) and asked what the boy was thinking that he would fish. The correct answer should be the fish, assuming that the boy has not yet pulled in his line to reveal the boot, which the viewer however can see.

Figure 2.3: An example of a True Belief Story, in which the children were shown a picture of a boy fishing, and in this example the boy has caught a fish. The children were then shown four pictures

(a fish, a boot, and two distracter objects) and asked what the boy was thinking that he would fish. The correct answer should be the fish, assuming that the boy believes what is really the case.

The test of non-mental representation (Zaitchik, 1990).

The test of non-mental representation (Zaitchik, 1990) is a test of physical rather than mental representation called “the false photo task” (Figure 2.4). The children were shown an old Polaroid camera with which they were allowed to take a photograph. Next the children were shown a girl doll in the bathroom of a toy house. The children were asked to take a photograph of the doll, using the old Polaroid and wait for the photo to develop. In the meantime, the experimenter showed the children that the mother doll comes in to move the girl doll into the bedroom of the house. The experimenter then asked the children three questions: a) where will the girl be in the picture when the photo is developed’ (test question); b) where was the girl when we took this picture? (memory question); c) where is the girl now? (reality question). All children have to answer the memory and the reality question correctly, in order to be scored for the test question.

a) **b)** **c)**

Figure 2.4: Scenarios used for the False Photo task. In the first scenario (a), the children were shown a girl doll in the bathroom of a toy house. Then, the experimenter took a picture with the old Polaroid camera (b). After that, the mother doll came and moved the girl doll from the first scenario (the bathroom) to the bedroom (c).

The test conservation of invisible particles after dissolution (Rosen & Rozin, 1993).

Children were given a small sample of sugar and the sugar was named as such. Children explored the substance by feeling, smelling and tasting. Children were then asked whether they liked the substance. After the child explored the powder and said that he/she likes it, two identical glasses containing 100 ml of water were introduced and named as water. One spoonful of the target substance (equal to 5g) was then stirred into one glass. The position of the target glass was randomised in each condition for each child. The glass of plain water was then also stirred with a new spoon for the same amount of time (4s). The choice-of-drink task directly measures the child's appreciation of the conservation of the taste property (e.g. sweetness) transferred to the solution by the child's indication if he/she would like or dislike each drink. The child was therefore asked for each of the pair of drinks "Is this one for you or does it really matter?

2.4 RESULTS

As shown in Figure 2.5, many children with ASD (n= 9; 45%) were ready to drink liquids that had been contaminated by insects. By contrast, the majority of TD children (n=24; 87%) and children with DS (n=9; 90%) demonstrated strong contamination sensitivity (X_2 (2, N = 57) = 7.329, p <.05).

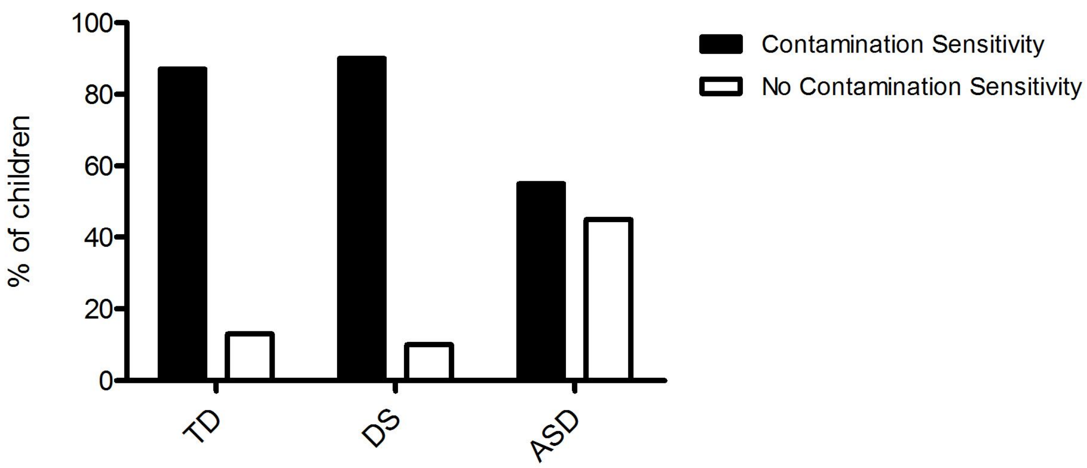


Figure 2.5: Percentage of Typically Developing children (TD), children with Down syndrome (DS) and children with ASD (Autism Spectrum Disorders) who refused to drink the contaminated juice (Contamination Sensitivity) and that agreed to drink the juice (No Contamination Sensitivity) in the classical contamination sensitivity task (Siegal and Share, 1990).

All the TD and the DS children were consistent in their responses to the contamination task and the contamination story. The children who claimed that the juice would make the boy sick in the story, justified their answer mainly by saying that the juice was dirty, poisoned or simply that it was not edible anymore after contact with an insect. The majority of children with autism who said that the juice was Ok to drink did not produce an answer for the story. An analysis of variance showed that the children with ASD with contamination sensitivity (*M*=76.27, *SE*=4.856) and the children with ASD without contamination sensitivity (*M*=68.67; SE =2.991) did not differ from the children with DS (*M*=78.89; SE=3.354) for verbal mental age, $F(2;26) = 1.635$, $p < .05$. Fisher exact probability test ($p > .05$) indicated that children with ASD who wanted to drink the juice did not differ either in their metarepresentational abilities nor in their knowledge about tiny, invisible particles in the liquid compared to the children that refused to drink the juice (Figure 2.6 and 2.7).

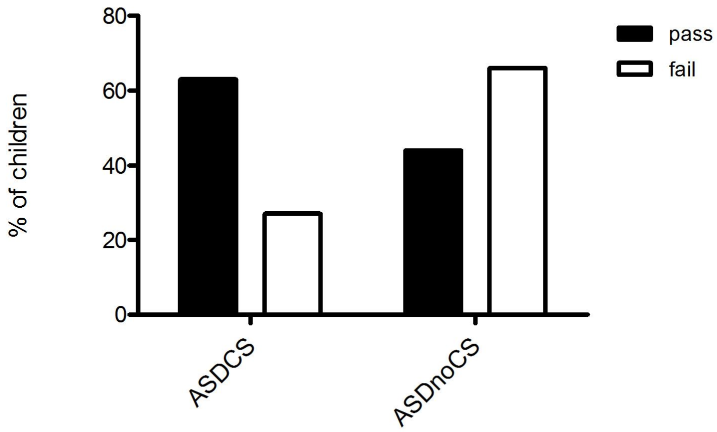


Figure 2.6: Percentage of children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) that pass or fail the test of meta-representational abilities in the false photo task.

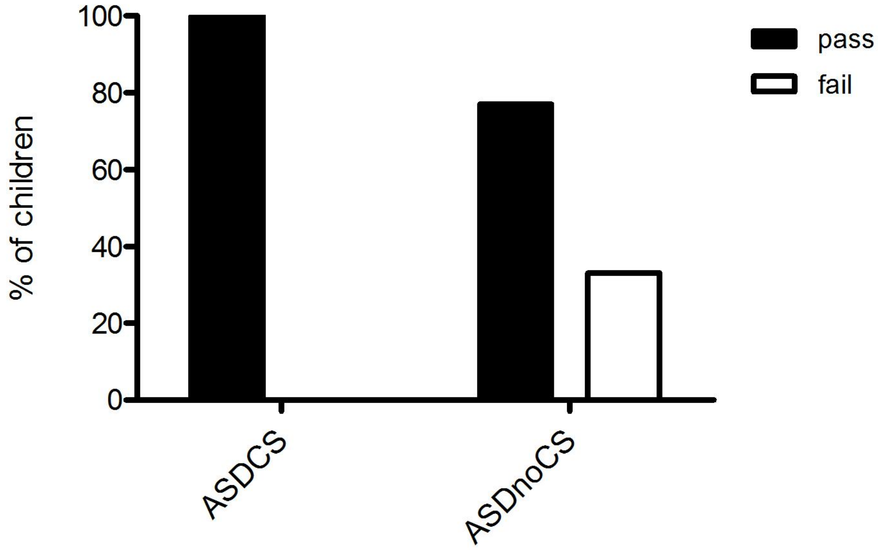


Figure 2.7: Percentage of children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) that passed or failed the test of conservation of invisible particles after dissolution.

Analysing in detail the specific characteristics of the ASD children in regard to contamination sensitivity (Table 2.1), we found that children who agreed to drink the juice were equivalent in chronological age, non-verbal mental age, verbal mental age and Theory of Mind to ASD children who refused to drink the juice.

	ASD with Contamination Sensitivity	**ASD without Contamination Sensitivity**	***t (18)***	***p***
Chronological age				
Mean	103.27	95.11	0.619	p > .05
SE	7.591	11.263		
Nonverbal Mental Age				
Mean	78.45	68	1.017	p > .05
SE	7.165	7.228		
Verbal Mental Age				
Mean	76.27	68.67	1.262	p > .05
SE	4.856	2.991		
Theory of Mind				
Mean	2.27	1.56	1.499	p > .05
SE	0.333	0.338		

Tab. 2.1: Individual differences in children with ASD in regard to contamination sensitivity.

2.5 Discussion

The results of this study indicated that a subgroup of children with ASD lack contamination sensitivity. In contrast, the majority of TD children and the majority of children with DS showed contamination sensitivity in response to drinks that had been contaminated by insects. These results are consistent with Kalyva et al. study (2009), which found that all 4- and 5-year old TD children and the majority of children with DS refused to drink the juice that had been in contact with an insect, while many children with ASD were prepared to drink liquids that had been contaminated by insects.

Differences between DS and ASD children in contamination sensitivity were confirmed in our study, in which the possible influence of different nationality present in Kalyva et al. study (2009) was controlled because both groups were from Italy. Moreover, the two groups of children were equivalent for verbal and non-verbal mental age. No other individual differences between children with ASD who lack contamination sensitivity and ASD children with contamination sensitivity were found. The two groups did not differ in verbal and non-verbal mental age, Theory of Mind abilities, non-mental representational abilities and knowledge about tiny, invisible particles that continue to exist in the liquids, even after dissolution. Differently from Kalyva et al. study (2009), children with ASDs who showed contamination sensitivity were not older in chronological age.

In summary, cognitive abilities don't seem to account for the differences in contamination sensitivity between ASD children and TD children and children with DS. Thus, these results point to the key role of access to language in promoting contamination sensitivity. To analyse this phenomenon in more depth, we specifically investigated auditory processing skills in Study 2, in relation to contamination sensitivity in ASD children and TD controls. Moreover, since disgust plays a central role in disease avoidance behaviour (Oaten, Stevenson & Case, 2009), and given the protracted debate over the role of GI symptoms in determining ASD symptomatology, we investigated whether and to what extent contamination sensitivity might be related to GI problems in ASD children.

Contamination sensitivity and auditory processing in children with ASD

3.1 Summary of the Chapter

Receptive and expressive deficits in language development in ASD have been attributed, in part, to abnormalities of auditory processing (Russo et al., 2009; Siegal & Blades, 2003). This Chapter illustrates how auditory processing skills influence the development of food contamination sensitivity in children with ASD. A group of children with ASD and a group of preschool TD children were tested for contamination sensitivity with a classical contamination task (Siegal and Share, 1990) and for auditory processing with the LiP test (Archbold, 1994) and a test of repetition of non-sense words. The results showed that children with ASD who were prepared to drink liquids that had been contaminated by insects had significantly lower auditory processing skills than children with ASD and controls who refused to drink contaminated drinks. These results are consistent with the auditory attention deficits well documented in ASD. Hence, auditory processing may play a significant role in the absence of contamination sensitivity in children with ASD in that such children may be precluded from attending to vital information from caregivers.

3.2 Introduction

Study 1 showed that many children with autism were ready to drink liquids that had been contaminated by insects. In contrast, the majority of TD children and children with DS demonstrated strong contamination sensitivity. The cognitive abilities measured in this study didn't seem to influence contamination sensitivity. These results tend to confirm the hypothesis that a restricted access to language and parent's conversations, who point out in everyday situations which foods are edible and which are not despite their appearance, may account for the lack of contamination sensitivity in children with autism. However, to what extent the lack of access to language might affect contamination sensitivity was not specifically investigated in Study 1.

On the basis of these considerations, the aim of Study 2 was to explore auditory processing skills in relation to contamination sensitivity in children ASD. Deficiencies in auditory processing have been detected in both perception and cortical encoding of speech sounds in ASD (see for example Kuhl et al., 2005; Russo et al., 2009; Simmons et al., 2011). The auditory abnormalities and consequent sensory deprivation might exacerbate the communication deficit in ASD, since successful communication relies on being able to both produce and process speech sounds in a meaningful manner (Siegal & Blades, 2003). If disgust is affected by language abilities, it is reasonable to predict that children with ASD who show higher auditory processing scores will be more aware about contamination than children with lower auditory skills.

Moreover, since disgust plays a central role in the mechanism of disease avoidance (Oaten, Stevenson & Case, 2009) and given the protracted debate over the role of GI symptoms in determining ASD symptomatology, we investigated the prevalence of GI problems in ASD children in relation to contamination sensitivity. The sense of disgust in parents was also considered, as measured by the Disgust Sensitivity Scale (Haidt, McCauley and Rozin, 1994) revised by Olatunji, Williams, Tolin, Sawchuck, Abramowitz, Lohr, et al. (2007). Parents were also interviewed about their perception of the sense of disgust and contamination in their children.

3.3 METHOD

Participants

23 children with ASD (18M; 5F), aged between 4 and 14 years (*M* = 8.1 years; *SD*=32), and 27 TD children (14M; 13F), aged between 3.7 years and 5.2 years (*M* = 4.3 years; *SD*=5.34) participated at the study. Children with ASD were delayed in development and therefore they were older in chronological age compared to TD children. They were diagnosed by expert clinicians on the basis of the DSM-IV criteria and the ADOS (Lord et al., 1999). The TD children were recruited in public schools in Cagliari (Italy) while ASD children were recruited through referrals in a Centre for Pervasive Developmental Disorders in Cagliari (Italy). 20 children with ASDs took part in Study 1 while the TD children participated at the study for the first time.

Measures

All the participants were tested for contamination sensitivity with the task developed by Siegal & Share (1990), in which the experimenter offered to the child some juice, in the course of natural conversation during their snack time. Then, an insect float on top of the juice and the experimenter told to the child: "Here's some juice. Oh! It has a cockroach floating on top". The experimenter removed the insect without a trace and asked, "Is the juice OK or not OK to drink?". The continued by telling the children a story in which the task will be to evaluate another's responses to contamination.

Moreover, they were tested for auditory processing abilities and for, with the following tools :

- ***Test for auditory processing: The Italian version of the Listening Progress Profile (LIP) developed by Sue Archbold (Nottingham Paediatric Cochlear Implant Program, 1994).***

The Listening Progress Profile (LiP) measures a range of early listening skills from the first response to environmental sounds and the first response to voice, through to discrimination of environmental sounds and discrimination of voice, to identification of the child's own name (Nikolopoulos, Wells & Archbold, 2000; Nikolopoulos, O'Donoghue, Robinson, Gibbin, Archbold & Mason, 1997). The LiP test consists of 21 subtests, hierarchically ordered, that can be grouped into three main categories as follows: detection, discrimination, identification. According to Erber (1982), the abilities to detect, discriminate and identify sounds and voices are fundamental prerequisites for the development of language recognition and understanding. The detection subtests evaluate the ability to perceive a specific type of sound (sounds or voices). The discrimination subtests investigate the ability to distinguish between two kinds of sound or between two features of the sound, like the rhythm, the timbre and so on. Finally, the identification subtests assess the ability to recognize a specific class of sounds compared to others.

In the LiP test, the children are exposed to environmental noises and their reactions are scored on a level from 0–2 (0 being never, 2 being always). The children are shown pictures and asked to point at the one he/she believes is the correct answer. For example, a child is presented with the sounds of two different musical instruments and observed as to whether he/she hears the instruments (detection) and if so is able to discriminate between the two instruments (discrimination). An identification task would be to recognize his/her own name. The inter-observer reliability of LiP as a measure of auditory perception has been formally validated and has shown high levels of agreement between different observers (Archbold, 1994).

For the purpose of Study 2, we selected five subtests of the LiP test, as follow: two subtests of detection of environmental sounds (the "GIOCO CON OCA, PESCE E CANE " and the "GIOCO DEL TAMBURO"), that assess the ability to detect environmental sounds; the subtest of "GIOCO CON OCA, PESCE E CANE, in which the experimenter not only observed the reaction of the child to the sounds (detection) but also asked the child to find or to name a specific sound (for example: "Find the duck!"); one subtest of detection of the human voice (the "GIOCO DELL'OCA"), that evaluates the ability to perceive the human voice; one subtest of discrimination of human voice (the "GIOCO DEI CINQUE LUPI"), that explores the ability of the children to discriminate between the Ling sounds: AA, II, OO, SH, SS. The Ling sounds are characterized by audiometric properties whose spectrum is considered to be the basis of the development of human language. In particular, the ability to discriminate the Ling sounds requires specific perceptive and cognitive abilities (detection, discrimination and recognition) that are fundamental to distinguish the segmental properties of the language, like syntax and semantic. Moreover, we also administered a subtest of identification of two words ("CIELO" and "FINESTRELLA"), assessed through the ability of the child to repeat the word after it had been heard from a computer. All the subtests have been administered with an interactive CD-ROM, which is meant to improve the motivation of the children to participate in the test thanks to a series of coloured and animated pictures and a number of visual and auditory rewards. Each subtest scored between 0 to 2, as follows:

- 0 = no sign of detection/discrimination/identification of any sound;
- 1 = sign of detection/discrimination/identification of some of the sounds;
- 2 = sign of detection/discrimination/identification of all the sounds.

The maximum score for all the subtest was 12, since the first subtest was coded twice: once for detection and once for identification.

– ***Standardized Nonsense Words Repetition task (Cornoldi, Miato, Molin, Poli, 1985).***

This is a phonemic discrimination task that requires to the children to repeat nonsense words of 1-4 syllables, right after that they have been stated by the experiment one by one. Phonemic discrimination is crucial for language manipulation, which plays a fundamental role in language acquisition and oral language (Bishop, North, & Donlan, 1996). Besides the ability of phonemic discrimination, the repetition of novel words implies an immediate memory of phonemes and non-words. This memory, defined by Baddeley (1996) as phonological working memory or the phonological loop, is fundamental for auditory comprehension because it temporarily stores novel phonological input, while a number of cognitive processes take place in order to understand the oral language (Baddeley, Gathercole, & Papagno, 1998). The standardized NonsenseWords Repetition task (Cornoldi, Miato, Molin, Poli, 1985) comprises a list of non-words (sequences of letters that do not form words), characterized by growing levels of complexity, as follows:

- 5 syllables: *BA, PUN, GLI, STRA, BLIZ*

- 5 disyllabic non-words of 5 letters: *NANTA, RORDO, VEVRE, SESPE, LOLCO*

- 5 disyllabic non-words of 6 letters*: NONTRO, SESTRE, SASFRA, LILTRI, MIMBRI*

-5 trisyllabic non-words: *PRUSTELA, FRANCITRA, STROMAFIO, TASTOLA, BRISTEGO*

-5 quadrisyllabic non-words: *PASTOMETRO, ANTRIVANO, DULCABRITE, STOPSONITE, UNDOCISTE*

The sequences of letters that form the non-words in this test have been selected as having a high rate of occurrence in the Italian language. The task was scored by assigning one point for every syllable stated correctly. A wrong accent was scored as an error. The maximum score was 60.

As in Study 1, all the children were tested for contamination sensitivity with a classical behavioral task (Siegal and Share, 1990). Children with ASD were tested for non verbal mental age with the Leiter International Performance Scale-Revised (Roid and Miller, 1997) and for verbal mental age with the Italian version of the Peabody Picture Vocabulary Test (Dunn & Dunn, 2000). They were also evaluated with a test of false and true beliefs (Woolfe, Want, & Siegal, 2002), a test of non-mental representation (Zaitchik, 1990) and a test of conservation of invisible particles (Rosen and Rozin, 1993).

The False Belief Task (Woolfe, Want, & Siegal, 2002).

The children were shown four ‘‘thought pictures’’: two in a false belief condition (FB), and the other two in a true belief condition (TB). The content of the pictures of FB and TB tasks was randomised across children. For each task, children were asked a belief and a reality question. They were scored as having passed the task if they answered both questions correctly. Each child therefore received a FB and a TB score ranging from 0 to 4.

The test of non-mental representation (Zaitchik, 1990).

The test of non-mental representation (Zaitchik, 1990) is a test of physical rather than mental representation called “the false photo task” . The children were shown an old Polaroid camera with which they were allowed to take a photograph. Next the children were shown a girl doll in the bathroom of a toy house. The children were asked to take a photograph of the doll, using the old Polaroid and wait for the photo to develop. In the meantime, the experimenter showed the children that the mother doll comes in to move the girl doll into the bedroom of the house. The experimenter then asked the children three questions: a) where will the girl be in the picture when the photo is developed’ (test question); b) where was the girl when we took this picture? (memory question); c) where is the girl now? (reality question). All children had to answer the memory and the reality question correctly, in order to be scored for the test question.

The test conservation of invisible particles after dissolution (Rosen & Rozin, 1993).

Children were given a small sample of sugar and the sugar was named as such. Children explored the substance by feeling, smelling and tasting. Children were then asked whether they liked the substance. After the child explored the powder and said that he/she likes it, two identical glasses containing 100 ml of water were introduced and named as water. One spoonful of the target substance (equal to 5g) was then stirred into one glass. The position of the target glass was randomised in each condition for each child. The glass of plain water was then also stirred with a new spoon for the same amount of time (4s). The choice-of-drink task directly measures the child’s appreciation of the conservation of the taste property (e.g. sweetness) transferred to the solution by the child’s indication if he/she would like or dislike each drink. The child was therefore asked for each of the pair of drinks “Is this one for you or does it really matter?

GI problems were assessed via the procedure used by Nikolov et al. (2009), which evaluated GI problems in a large, well characterized sample of children with Pervasive Developmental Disorders. The experimenter administered a series of questions to the parents, aimed at determining whether the child has or had in the past any GI problems (Appendix 1). Specifically, it the parents were asked if:

- any GI problem was present now or in the past;
- the GI problem had been brought to the attention of a clinician;
- the GI problems is or had been under treatment.

At least one of those conditions were sufficient to rate the child has having GI problems (Appendix 1).

The Disgust-scale Revised (Haidt, McCauley, Rozin, 1994).

Parents were administered the Disgust Sensitivity Scale Revised, as a measure of their sensitivity to core disgust elicitors, animal reminders and contamination disgust (Appendix 2). The original Disgust Sensitivity Scale (Haidt, McCauley and Rozin, 1994) was a 32 item scale, first published in 1994. This version had 8 subscales with four items per subscale. The items run in this order: food, animals, body products, sex, envelope violations, death, hygiene, and magical thinking. It was designed to be usable by widely varying populations, so the response scale was kept simple: true/false in the first half, and a 3-point disgust rating scale on the second half. These 8 subscales did not have sufficiently high reliability to be considered distinct individual difference measures; they were included for exploratory purposes, to make sure that they covered the full range of disgust elicitors, and to explore differences among populations (e.g., nurses, people with obsessive compulsive disorder, women versus men, etc.).

Olatunji et al. revised the original Disgust Sensitivity Scale DS-R), and proposed a new version with 4 main improvements (Olatunji, Williams, Tolin, Sawchuck, Abramowitz, Lohr, et al., 2007). First, the items were reduced from 32 to 25, based on Olatunji et al.'s reanalysis of which items were contributing to total score. Second, the number of subscales was reduced from the original 8 (most of which had low reliability) to just the three subscales that show up consistently in factor analyses: Core disgust (including food, animals, and body products), Animal-reminder disgust (death and envelope violations) and contamination disgust (concerns about interpersonal transmission of essences). All three subscales showed alphas above 70. Third, the scales were changed (from true-false in part 1 and 3-point ratings in part 2) so that all items now rated on 5-point scales (0-4). Finally, the DS-R included two "catch" questions which allowed the tester to identify and remove people who are either not paying attention or not taking the task seriously. The scores range from 0 to 100.

The parents were also asked to describe if their children showed any contamination sensitivity responses in daily life, through a questionnaire based on the first subscale of the DS-R, developed by Haidt, McCauley, & Rozin (1994). The questionnaire includes 9 questions about food rejection and contamination sensitivity, rated on 5 point scales, ranging from 0-4 (Appendix 1).

3.4 RESULTS

As shown in Figure 3.1, many children with ASD (*n*=10; 44%) were ready to drink liquids that had been contaminated by insects. By contrast, the majority of typically developing children (*n*=26; 96%) demonstrated strong contamination sensitivity (Fisher's exact test probability $p < .05$).

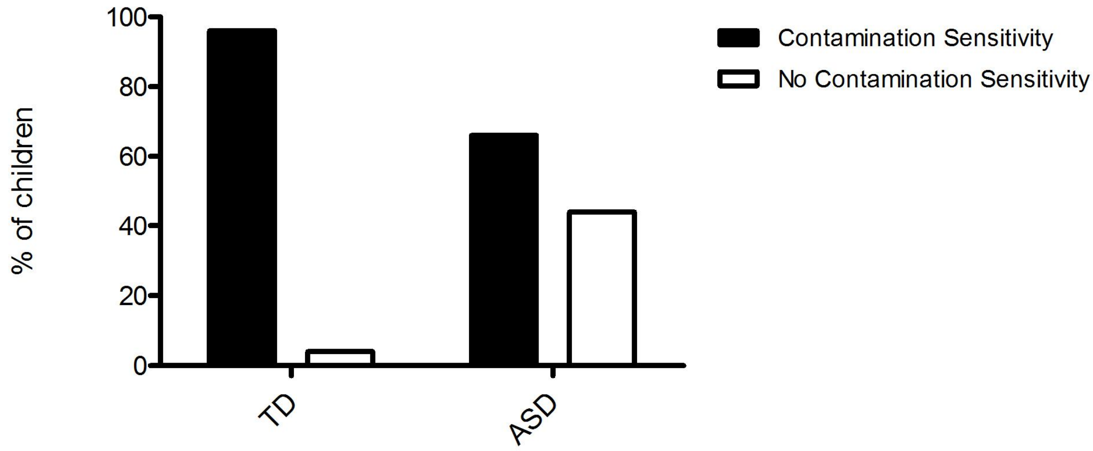


Figure 3.1: Percentage of Typically Developing children (TD) and children with ASD (Autism Spectrum Disorders) who refused to drink the contaminated juice (Contamination Sensitivity) and that agreed to drink the juice (No Contamination Sensitivity) in the classical contamination sensitivity task (Siegal and Share, 1990).

All the TD children were consistent in their responses to the contamination task and the contamination story. The children who claimed that the juice would make the boy sick in the story, justified their answer mainly saying that the juice was dirty, poisoned or simply that it was not edible anymore after the contact with an insect. The majority of children with autism who said that the juice was OK to drink did not produce an answer for the story. In order to verify a possible role of auditory processing in contamination sensitivity, we explored the differences in auditory competence between typically developing children and children with autism with and without contamination sensitivity. The one way ANOVA showed significant differences between the three groups in auditory processing skills, respectively in sound perception, $F(2;48) = 31.740$, $p < .05$ (Figure 3.2) and in the repetition of non-words, $F(2;48) = 10.900$; $p < .05$ (Figure 3.3). In sound perception, a post hoc analyses indicated that the average number of sounds perceived was significantly lower in children with ASD who lack contamination sensitivity (*M=9; SD*=1.94) compared to controls (*M*=11.73; *SD*=0.452) and to children with autism with contamination sensitivity (*M*=11.77; *SD*=0.599). In the repetition of nonsense words, a post hoc analyses revealed that the children with ASD who lack contamination sensitivity (*M*=35; *SD*=13.379) were lower than controls (*M=48; SD*=5.522) and lower than children with ASD with contamination sensitivity (*M=49; SD*=7.960).

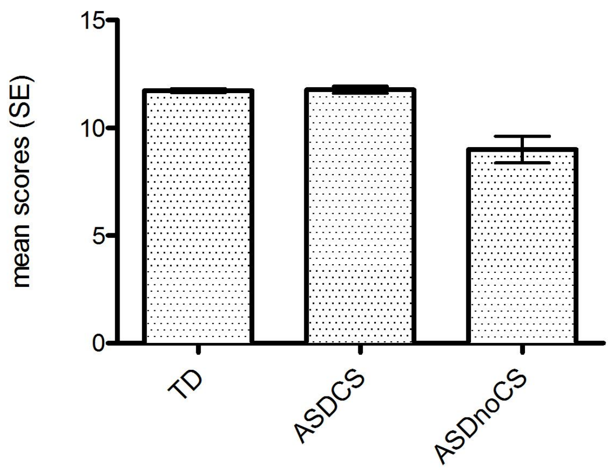


Figure 3.2: Mean scores in the sound perception task (LiP test) - error bars represent standard errors - in Typically Developing children (TD), children with ASDs with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS).

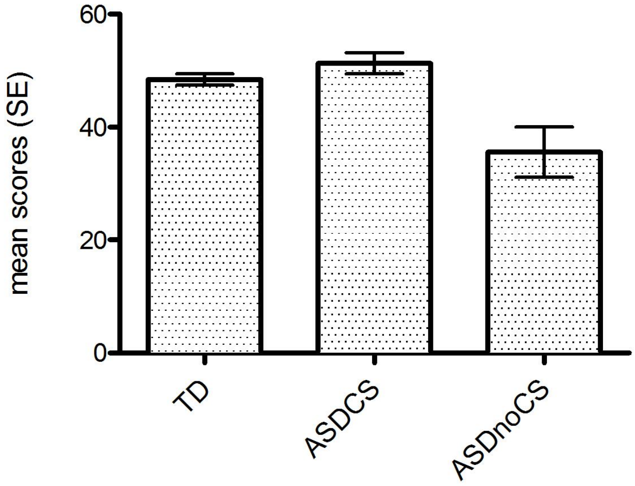


Figure 3.3: Mean scores in the repetition of non-sense words - error bars represent standard errors - in Typically Developing children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS).

Fisher exact probability test ($p > .05$) indicated that children with ASD who were willing to drink the juice did not differ in two abilities crucial for contamination sensitivity: they were as good as children with autism who did not agree to drink the contaminated juice both in metarepresentational abilities (Figure 3.4) and in understanding of the concept of invisible particles after dissolution (Figure 3.5).

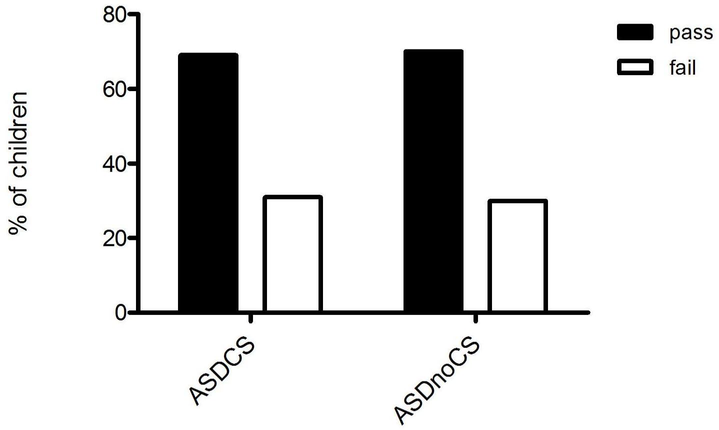


Figure 3.4: Percentage of children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) that pass or fail the test of meta-representational abilities in the false photo task.

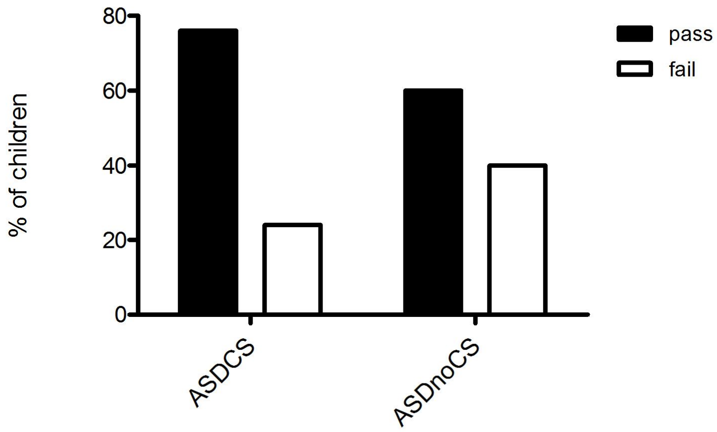


Figure 3.5: Percentage of children with ASDs with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) that pass or fail the test of conservation of tiny, invisible particles after dissolution.

When we evaluated the incidence of GI symptoms (Figure 3.6) in relation to contamination sensitivity, Fisher exact probability test revealed that ASD noCS were not different from TD (*p*=0.1550), ASD noCD were not different from ASD CS (*p*=0.4536). Moreover, ASD CS were not different from TD (*p*=0.3211).

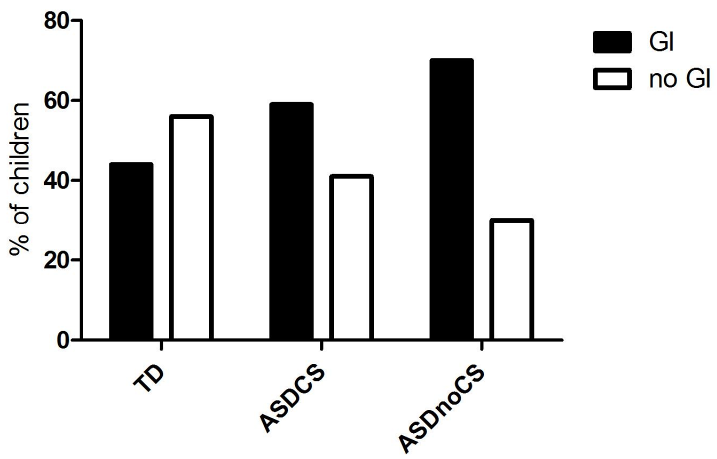


Figure 3.6: Percentage of children with GI symptoms in the three groups: Typically Developing children (TD), children with ASD with Contamination Sensitivity (ASD CS) and children with ASD without Contamination Sensitivity (ASD noCS).

In order to verify the existence of any possible intervening variable in auditory processing performance, we analyzed in detail the following characteristics of the ASD children in regard to contamination sensitivity (Table 3.1): chronological age, non-verbal mental age, verbal mental age and Theory of Mind. No individual differences were found between the two groups.

	ASD with Contamination Sensitivity	***ASD without Contamination Sensitivity***	***t (df=26)***	***p***
Chronological age				
Mean	101	92.40	0.685	p > .05
SE	7.651	10.508		
Nonverbal mental age				
Mean	79.92	71.30	1.009	p > .05
SE	5.785	6.273		
Verbal mental age				
Mean	76	67.20	1.744	p > .05
SE	4.221	1.645		
Theory of Mind				
Mean	1.85	1.20	1.258	p > .05
SE	1.281	1.135		

Tab. 3.1: Individual differences in children with ASD in regard to contamination sensitivity.

Moreover, the three groups did not differ in the DS-R scores of the principal caregiver, $F(2;35) = 0.186$; $p > .05$.

Interestingly, there were differences between children in the disgust sensitivity reported by their parents on the questionnaires, $F(2;38) = 9.111$; $p < .05$. A post-hoc test indicated that children with ASD without contamination sensitivity were described as having lower levels of disgust sensitivity by their parents (M=11.14; SD=6.256) compared to TD controls (M=23.91; SD=7.628) and to ASD CS (M=19.92; SD=5.744).

3.5 Discussion

The results of Study 2 confirm the existence of a subgroup of children with ASD that lack contamination sensitivity. This is in contrast with the TD children, that showed a strong sense of contamination sensitivity with the exception of only one child.

When we compared the ASD noCS with TD and ASD CS in auditory processing skills, we found that children with ASD who lacked contamination sensitivity were lower in auditory processing skills, both in sound perception and in the repetition of non-words, in comparison to TD children and children with ASD who showed contamination sensitivity.

Differences in contamination sensitivity in ASD were not related to differences in chronological age, verbal and non-verbal mental age, Theory of Mind, non-mental representation and the ability to reason about tiny, invisible particles. Moreover, the differences in contamination sensitivity couldn't be explained in terms of differences in parent's perception of disgust. The principal caregivers of the children in the three groups (TD, ASD CS, ASD noCS) did not differ in disgust sensitivity as measured by the Disgust Sensitivity Scale. However, children with ASD who lacked contamination sensitivity were perceived by their parents as less sensitive to disgust and contamination in respect to the other two groups.

Taken together, all these results seem to indicate that auditory processing deficits play a key role in putting children with ASD at risk of not developing an adequate sense of contamination. When we investigated the possible relationship between GI symptoms and contamination sensitivity, there were not significant differences between the three groups. These results about GI symptoms seems to indicate that, interestingly, the subgroup of children with ASD lacking contamination sensitivity in behavioral task and rated by the principal caregiver as less sensitive to disgust and contamination is not characterized by an increased incidence of GI diseases (which are known to be caused in some cases by poor hygiene or by unhealthy behaviors).

In summary, Study 2 confirmed a lack of contamination sensitivity in a subgroup of ASD children, previously found in Study 1. Moreover, as in Study 1, it confirms that contamination sensitivity is independent from cognitive abilities in ASD children. Moreover, this study demonstrated a deficit in auditory processing skills in children with ASD that lack contamination sensitivity. Thus, these results seem to further support the hypothesis of a prominent role of access to language and conversation in the development of this ability.

However, this study was limited because it involved only a behavioural task to assess contamination sensitivity. Classical contamination sensitivity tasks (for example

Siegal and Share, 1990), as the one used in this study, evaluates behavioural reactions to contaminants by asking children to choose to drink a juice that has been contaminated or not. While this task has been widely used in typical populations, it has been used only recently with children with autism so its sensitivity for atypical children still has to be established. It is possible that those children with ASD who were willing to drink the contaminated juice actually had an implicit sense of disgust and contamination but simply they misbehaved in the classical contamination task. Is it the case that they didn’t feel disgusted at all observing an insect floating on top of a juice or is it that they could not refrain from drinking?

With this question in mind, we developed two more studies in which we employed a non verbal experimental paradigm to test an implicit reaction to contaminants: a preferential looking task implemented with eye-tracking.

4.1 Summary of the Chapter

Contamination sensitivity, which typically emerges at around 4yrs of age thanks to a combination of cognitive abilities and social learning processes, seems to be particularly impaired in children with ASD (Kalyva, Pelizzoni et al., 2009). However, since contamination sensitivity in children with ASD has only been investigated through behavioral studies, to what extent children with ASD who lack explicit contamination sensitivity in behavioral tasks are implicitly sensitive to disgust elicitors needs to be specifically investigated. In this Chapter, we evaluated implicit contamination sensitivity in two subgroups of ASD children, that respectively lacked explicit contamination sensitivity (ASD noCS) and that showed explicit contamination sensitivity (ASD CS) in a classical contamination task (Siegal and Share, 1990), and a group of TD controls, using an eye-tracking preferential looking paradigm. The two subgroups of children with ASD were selected by the means of a one-to-one matching procedure for verbal, non-verbal and chronological age. The results showed that TD and ASD CS children had a looking preference for an uncontaminated drink in sharp contrast to children with ASD who did not possess contamination sensitivity. These results show that children with ASD who lack explicit contamination sensitivity also lack an implicit sensitivity to disgust elicitors, highlighting the importance of pairing behavioral tasks with eye-tracking measures to reliably assess clinical populations.

4.2 Introduction

Study 2 demonstrated a lack of contamination sensitivity in children with ASD. It demonstrated also that children with ASD who lacked contamination sensitivity had lower auditory processing skills, compared to TD controls and to children with ASD who showed contamination sensitivity. These results indicated that the ability to attend to language might play an important role in the development of contamination sensitivity. However, while classical behavioural tasks have been widely used in typical populations, they have been used only recently with children with ASD so their reliability for atypical children needs to be further investigated. As a consequence, we wondered whether children with ASD who agreed to drink contaminated juice don't actually have any contamination sensitivity at all or, on the contrary, whether they are characterized by an implicit sense of disgust which they can't express in behavioural tasks.

Indeed, classical contamination sensitivity tasks (for example Siegal and Share, 1990) evaluate behavioural reactions to contaminants by asking children if it's OK to drink a juice that has been contaminated by an insect. These tasks are meant to be behavioural, insofar as the behaviour of the child tells us something about his/her understanding of contamination sensitivity: if a child refuses to drink the juice, it means that he/she understands the contaminating nature of the insect. However, this kind of task evaluates an explicit understanding of contamination sensitivity, which implies a number of cognitive and linguistic abilities. First, in order to be successful in this task, a child needs to communicate his/her choice to the experimenter, either verbally or non verbally. Second, the child needs to inhibit his/her impulse to drink a liked drink (the juice) because of his/her knowledge that the juice has been contaminated, which requires quite intact executive control.

Thus, contamination sensitivity in children with ASD might be masked by their deficits in language, communication and social interaction (Tager-Flusberg, 1996) or by a deficit in executive functioning (Hill, 2004). A non-verbal task might therefore provide a more reliable measure of contamination sensitivity, because it does not require either a verbal judgement or a voluntary choice between two alternatives.

On the basis of these considerations, we developed a non-verbal paradigm to evaluate contamination sensitivity in children with ASD. The classical behavioural task was transposed onto a video, and an eye-tracker was used to monitor the behaviour of the participants. We used two measures that might respectively avoid socio-communication and executive functioning deficit in children with ASD: preferential looking and variations in pupil size. The first one considers the length of observation as an index of "preference" towards a contaminated juice *vs* a non-contaminated juice. The second one is an involuntary, unconscious response to a contaminant, controlled by sub-cortical components of the nervous system. Its variation is completely independent of the frontal cortex and therefore it's not related to executive functioning.

We predicted that children with ASD who lack contamination sensitivity would show neither a looking preference toward an uncontaminated juice nor a pupillary reaction to watching an insect floating on top of a glass of orange juice.

4.3 METHOD

Participants

30 TD children (15 boys), ranging in age from 48 to 79 moths (mean age=61 months, SD=1.593) and 38 children with ASD (31 males), diagnosed by expert clinicians on the basis of the DSM-IV criteria and the ADOS (Lord et al., 1999), aged between 3.10 years and 14 years (mean age=8.1 years; SD=31,924) participated at the study. Children with ASDs were delayed in development and therefore they were older in chronological age compared to TD children. The TD children were recruited in public schools in Cagliari (Italy) while ASD children were recruited through referrals at a Centre for Pervasive Developmental Disorders in Cagliari (Italy). 14 children with ASD took part in study 1, 2 children with ASD took part in study 1 and 2 while the TD children participated at the study for the first time.

Setting

The study was set in a small room. To ensure that the lighting conditions were the same for all of the subjects, we had the blinds lowered and the neon light on. The participants were seated on a chair at a distance of about 68 cm (27 inch) from the screen. The stimuli were presented with a Tobii eye tracking 17” TFT flat screen (resolution 1280x1024 pixels), activated and controlled through a laptop computer by an experimenter, sitting behind a screen in order to be hidden from the view of the participants. Responses were recorded at 60 Hz using a Tobii T60 eye tracker (Tobii technology), with an accuracy of 0.5 degrees of visual angle.

Materials

The videos were presented with the Tobii T60 Eye Tracker. The tests for auditory processing were administered with a multimedia CD while the behavioural tasks were presented with pictures, small objects and toys.

Procedure

First, the participants were tested for explicit contamination sensitivity with the task developed by Siegal & Share (1990). In the explicit contamination sensitivity task (Siegal & Share, 1990), the experimenter offered to the child some juice, in the course of natural conversation during their snack time. Then, an insect float on top of the juice and the experimenter told to the child: “Here’s some juice. Oh! It has a cockroach floating on top”. The experimenter removed the insect without a trace and asked, “Is the juice OK or not OK to drink?”. The continued by telling the children a story in which the task will be to evaluate another’s responses to contamination.

The children with ASD with explicit contamination sensitivity (ASD CS) were matched one-to-one with the ASD children without explicit contamination sensitivity (ASD noCS) for chronological age and for non-verbal and verbal mental-age, measured respectively by the Leiter International Performance Scale-Revised (Roid and Miller, 1997) and the Italian version of the Peabody Picture Vocabulary Test (Dunn & Dunn, 2000). The children that did not match for these three criterions were not included in the rest of the procedure. After few days, the two subgroups of ASD children (ASD CS and ASD noCS) and the TD controls were tested for implicit contamination sensitivity with an eye-tracking paradigm, involving a set of videos developed for the purposes of this study.

Each participant was invited to sit in an adjustable chair in front of the computer screen. All the participants successfully completed a calibration phase before the testing, during which the eye tracker was tuned to the eyes of each participant using a preinstalled calibration tool: a moving colourful red dot on a light gray background was presented in 5 predefined calibration points (the corners and the middle of the screen). Participants were told to follow the dot with their eyes as it was moving across the screen. Once the subject followed the calibration image and the eye tracker picked up enough observation data in all of the 5 points, the calibration was completed. After the calibration phase, the participants were told to watch to the screen, where some videos would appear. Each participant was instructed to look at a fixation cross at the center of the screen. Twenty seconds from the onset of the fixation point the first video was delivered.

Each child was tested with a sequence of videos, which represent a classical contamination task (Siegal & Share, 1990) and a manipulations of this task, in which the contaminant was substituted by an edible substance (sugar). The two test conditions followed two habituation videos, in which an adult poured some juice into two glasses in front of a child, one on the left and one on the right, then asked the child : “Which one is for you?”. Then, after 5 seconds, the child reached for one of the two glasses (Fig. 4.1). The habituation videos were meant to elicit a clear expectation about the course of the interactions: namely, that the child would grasp one of the two glasses. In one of the habituation videos, the child grasped the juice to the left while in the other habituation video the child grasped the juice to the right. The order of the direction of the grasping (left *vs* right) was counterbalanced between subjects.

Figure 4.1: An example of habituation video used in Study 3

1) 

2) 

3) 

4) 

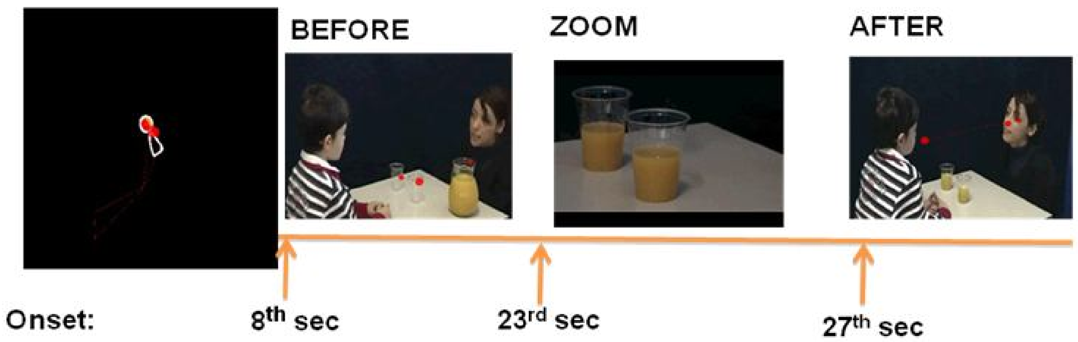
5) 

6) 

In the contamination condition (Siegal & Share, 1990), the video showed an adult pouring some juice into two transparent glasses (the same quantity in each glass). Then, an insect floated on top of one glass of juice and the adult said: “Oh, there’s an insect!” and removed it without a trace. Then, she asked to the child: “Which one is for you?”. The language accompanying the actions was meant to be simply descriptive, in order to make the content of the videos as explicit as possible for the children. In the sugar condition, the adult poured some juice into two transparent glasses (the same quantity in each glass). Then, she put one spoonful of sugar in one of the glasses and she said: “Oh, I put in some sugar!” and removed the empty spoon. Then, she asked to the child: “Which one is for you?”. The order of presentation of the two conditions was counterbalanced between subjects, as well as the position of the stimuli into the glasses (left *vs* right) and the direction of the grasping of the child in the baselines (left *vs* right). The display luminosity and the background colours were absolutely equivalent in the two tasks to control for light effects on pupil response.

The video used had a stable structure, in which there was defined sequence of events and a defined language content before and after a zoom, aimed to highlight the content of the glasses in the two conditions (Figure 4.2). It was the zoom that was manipulated into the two conditions: contamination (an insect floated on top of the juice) and no contamination (a spoonful of sugar was introduced into the juice).

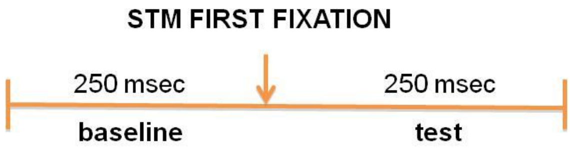
Figure 4.2: Timeline of the videos presented to the children.



We decided to manipulate the zoom to measure pupil reaction for three specific reasons: there are stable and not really meaningful events before and after the zoom, which prevent fluctuations in pupil diameter due to cognitive load or to semantic elaboration of the stimulus; there is stable luminance level which prevents fluctuation of pupil diameter due to changes in light level; and there is a relatively short time period pre zoom which prevents fluctuations of attention due to fatigue or boredom.

500ms interval of the zoom was selected for analysis, anchored on the first stimulus fixation (Figure 4.3). We selected a 500ms interval because it is known from previous studies that task-evoked pupillary responses occur quite quickly (300-500 msec) and dissipate equally quickly when processing ceases (Beatty, 1986). This brief time interval is appropriate for some processes in social psychology, such as affect and impression formation. A short latency response should be indicative of an implicit understanding of the stimuli, whilst a longer latency response would be indicative of a semantic elaboration of the stimuli, which enhances the richness of the phenomena (Beatty, 1986).

Figure 4.3: 500ms interval time course, anchored around stimulus (STM) first fixation.



To evaluate the possible role of individual differences in contamination sensitivity, children with ASD were tested for auditory processing with the LiP test (Archbold, 1994) and with a list of standardized non-sense words (Cornoldi, Miato, Molin, Poli, 1985) and for Theory of Mind abilities (Woolfe, Want, & Siegal, 2002). The order of presentation of these tasks was counterbalanced between subjects.

Test for auditory processing: The Italian version of the Listening Progress Profile (LIP) developed by Sue Archbold (Nottingham Paediatric Cochlear Implant Program, 1994).

The Listening Progress Profile (LiP) measures the ability to detect, discriminate and identify sounds or voices. For the purpose of this study, we administered two subtests of detection of environmental sounds; a subtest of detection and identification; one subtest of detection of the human voice; one subtest of discrimination of the Ling sounds: AA, II, OO, SH, SS. The Ling sounds are characterized by audiometric properties whose spectrum is considered to be the basis of the development of human language. Moreover, we also administered a subtest of identification of two words. All the subtests have been administered with an interactive CD-ROM, which is meant to improve the motivation of the children to participate in the test thanks to a series of coloured and animated pictures and a number of visual and auditory rewards. Each subtest scored between 0 to 2, as follows:

- 0 = no sign of detection/discrimination/identification of any sound;
- 1 = sign of detection/discrimination/identification of some of the sounds;
- 2 = sign of detection/discrimination/identification of all the sounds.

The maximum score for all the subtests was 12, since one subtest was coded twice: once for detection and once for identification.

Standardized Nonsense Words Repetition task (Cornoldi, Miato, Molin, Poli, 1985).

The standardized NonsenseWords Repetition task (Cornoldi, Miato, Molin, Poli, 1985) comprises a list of non-words (sequences of letters that do not form words), characterized by growing levels of complexity. The sequences of letters that form the non-words in this test have been selected as having a high rate of occurrence in the Italian language. The task was scored by assigning one point for every syllable stated correctly. A wrong accent was scored as an error. The maximum score was 60.

They were also evaluated for Theory of Mind abilities with tests of false and true beliefs (Woolfe, Want, & Siegal, 2002), a test of non-mental representation (Zaitchik, 1990) and a test of conservation of invisible particles after dissolution (Rosen and Rozin, 1993). The order of presentation of these tasks was counterbalanced between subjects.

The False Belief Task (Woolfe, Want, & Siegal, 2002).

The children were shown four ‘‘thought pictures’’: two in a false belief condition (FB), and the other two in a true belief condition (TB). The content of the pictures of FB and TB tasks was randomised across children. For each task, children were asked a belief and a reality question. They were scored as having passed the task if they answered both questions correctly. Each child therefore received a FB and a TB score ranging from 0 to 4.

The parents of all the children were administered the Disgust Sensitivity Scale Revised (Haidt, McCauley, & Rozin, 1994), as a measure of sensitivity to core disgust elicitors, animal reminders and contamination disgust. Parents were also asked to describe possible GI symptoms of the children, which were evaluated on the basis of Nikolov et al. (2009) criteria as in Study 2. Moreover, they were asked to describe if their children showed any contamination sensitivity in daily life, through a short questionnaire based on the first subscale of the Disgust Scale Revised, developed by Haidt, McCauley, & Rozin, 1994.

Measures of preferential looking

Measures of preferential looking were obtained by considering the looking time toward specific Areas of Interest (AOIs; Figure 4.4), which had the same dimensions in the two conditions:

- **contamination condition:** contaminated glass *vs* uncontaminated glass;
- **no contamination condition:** glass of juice with sugar *vs* glass without sugar.

Figure 4.4: Areas of Interest selected to be analysed in terms of looking time

Preferences for the AOIs were analysed by employing the following measures:

- **Fixation Count (FC):** the number of fixations within an AOI;

- **Observation Length (OL):** the total time in seconds for every time the person looked within an AOI, starting with a fixation within the AOI and ending with a fixation outside the AOI.

We compared the participants in the three groups for each of the two measures considered (FC and OL) by a 2x2x3 mixed between-within subject analysis of variance (fixed factor = GROUP: children with ASD CS, ASD noCS and TD children; random factors = AOI: glass with contaminant or sugar, clean glass; CONDITIONS: insect, sugar).

Data were further evaluated qualitatively with an estimation of the preference for one of the two glasses in the two conditions. The preference was defined as the difference in the looking time (Observation Length) between the glass with the insect or sugar and the clean glass, so that a positive delta would show a preference for the insect or for the sugar in each condition. The children who did not show any preference (equal observation length toward the two gaze targets) were excluded from this analysis. Moreover, the first glass at which the children looked at was considered.

Measure of pupil size

Pupillary responses were collected from the left eye and synchronized with the tasks. The pupil size, defined as the actual, external physical size of the pupil, was measured in mm and registered in miliseconds. The Tobii Eye Tracker uses an optical sensor which registers an image of the eyes that is used to calculate an eye model. This model allows for calculations of the pupil size by measuring the diameter of the pupil on the image and multiplying it with a scaling factor. In the eye model used by Tobii Eye Trackers, the pupil size is defined as the actual, external physical size of the pupil.

Pupil size, measured as the average dilation over the 250 ms preceding the onset of the stimulus, was subtracted from pupil size over the 250 ms following the stimulus onset, to produce pupil dilation difference scores. Pupil size was recorded every 17 msec. Average pupil size variation waveforms were obtained by aggregation of the data for each participant and condition (500 ms time course). Average size variation at 200 msec before stimulus onset was taken as a baseline and all pupil scores were normalized into difference scores from baseline.

4.4 RESULTS PART I: *Explicit contamination sensitivity*

All the participants were evaluated with the classical contamination sensitivity task, developed by Siegal & Share (1990). The results (Fig. 4.5) showed that all the TD children refused to drink a contaminated juice, while only the 53% of ASD children showed contamination sensitivity (Fisher's exact test probability $p<0.05$).

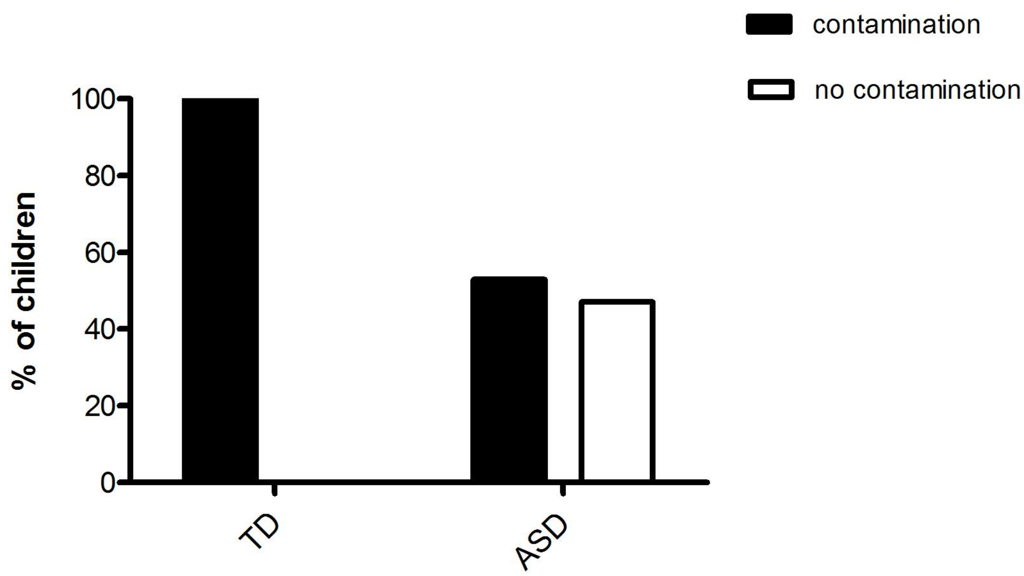


Figure 4.5: Percentage of Typically Developing children (TD) and children with ASD (Autism Spectrum Disorders) who refused to drink the contaminated juice (Contamination Sensitivity) and that agreed to drink the juice (No Contamination Sensitivity) in the classical contamination sensitivity task (Siegal and Share, 1990).

Following the explicit contamination sensitivity task, 15 children with ASD with explicit contamination sensitivity and 15 children with ASD without explicit contamination sensitivity were matched one-to-one for chronological age, verbal and non-verbal mental age (Table 4.1). 3 ASD children were excluded after the one-to-one matching because they did not match for chronological age. Some of the ASD children were excluded for other reasons: 2 children did not collaborate to the eye-tracker task; 3 did not come to the Hospital after the behavioral task to participate at the rest of the procedure.

	ASD CS n=15	***ASD noCS n=15***	*t* (28)	***p***
Chronological age				
Mean	106.60	87.23	1.674	p > .05
SE	6.829	8.713		
Nonverbal Mental age				p > .05
Mean	70.27	71.45	0.200	
SE	5.608	6.136		
Verbal Mental Age				
Mean	69.43	71.08	-0.151	p > .05
SE	2.941	2.598		

Table 4.1. Characteristics of ASD in relation to contamination sensitivity.

To investigate the possible role of individual differences in contamination sensitivity, children with ASD in the two subgroups (with and without contamination sensitivity) were tested for auditory processing skills and for Theory of Mind abilities. As shown in Table 4.2, the two subgroups significantly differ in auditory processing skills.

	ASD CS n=15	***ASD noCS n=15***	*t* (28)	***p***
Theory of Mind				
Mean	1.71	1.21	1.352	p > .05
SE	0.348	0.291		
Perception of sounds				
Mean	11.79	10.25	3.694	p < .05*
SE	0.136	0.583		
Non-words repetition				
Mean	47.71	40.27	2.673	p < .05*
SE	1.855	2.716		

Table 4.2. Individual differences in children with ASD in relation to contamination sensitivity.

4.5 RESULTS PART II: *Looking Preference*

Children's looking preference was analysed considering the numbers of Fixations (Fixation Count = FC) toward the two glasses in each condition separately (insect and sugar). To determine whether children had a preference for one of the two glasses, we computed a 3 (group: TD, ASD CS and ASD noCD) X 2 (glass: with/without insect) mixed-model ANOVA on the FC scores. For the insect condition, this analysis yielded a significant main effect of glass, $F_{1,57}$=6.040, $p < .05$, indicating that the participants discriminated between the two glasses and preferred one. There was not a main effect of group, $F_{1,57}$=0.292, $p > .05$, and there was not a significant effect of interaction glasses x group, $F_{2,57}$= 1.548, $p > .05$.

In the sugar condition, there was a significant main effect of glass, $F_{1,57}$=7.305, $p < .05$, indicating that the participants discriminated between the two glasses. There was not a significant main effect of group, $F_{1,57}$=1.870, $p > .05$, and no effect of interaction glasses x group, $F_{2,57}$= 1.366, $p > .05$ (Figure 4.6 and 4.7).

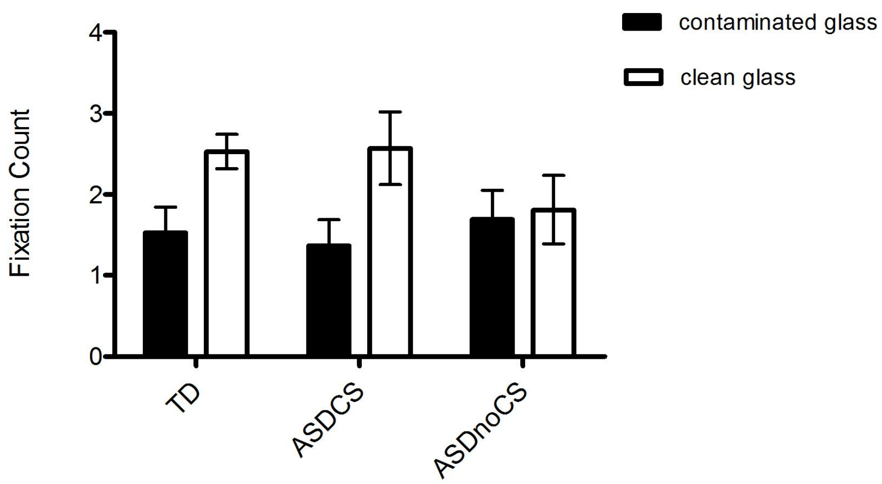


Figure 4.6: Fixation Count in the insect condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (contaminated *vs* clean). The contaminated glass was the glass containing the insect.

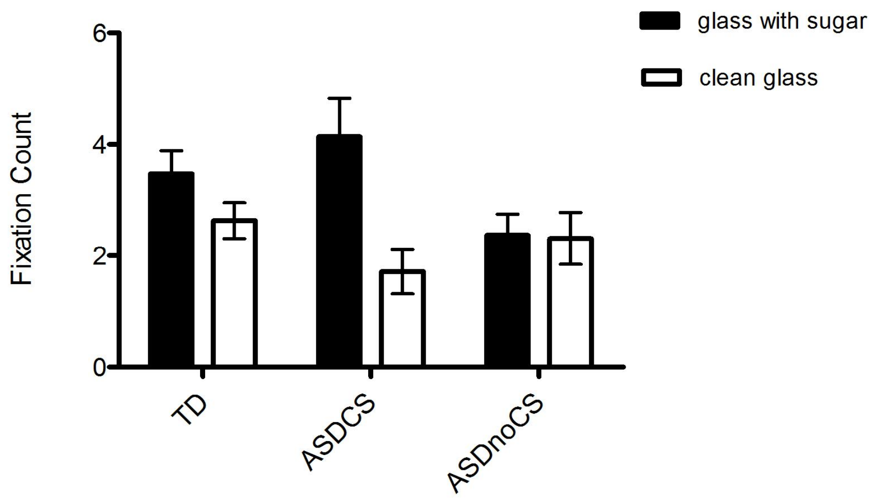


Figure 4.7: Fixation Count in the sugar condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (glass with sugar *vs* glass with pure juice). The clean glass was the glass in which only pure juice was present.

Then, we considered the length of observation (OL). A 3 (group: TD, ASD CS and ASD no CS) X 2 (glass: with and without external substance) mixed analysis of variance (ANOVA) computed for the insect condition revealed a significant main effect of glass, $F_{1,57}$=7.873, $p < .05$, , indicating that the participants discriminated between the two glasses and preferred one. There was no a significant effect of interaction glasses x group $F_{2,57}$= 0.681, $p > .05$ (Figure 4.8).

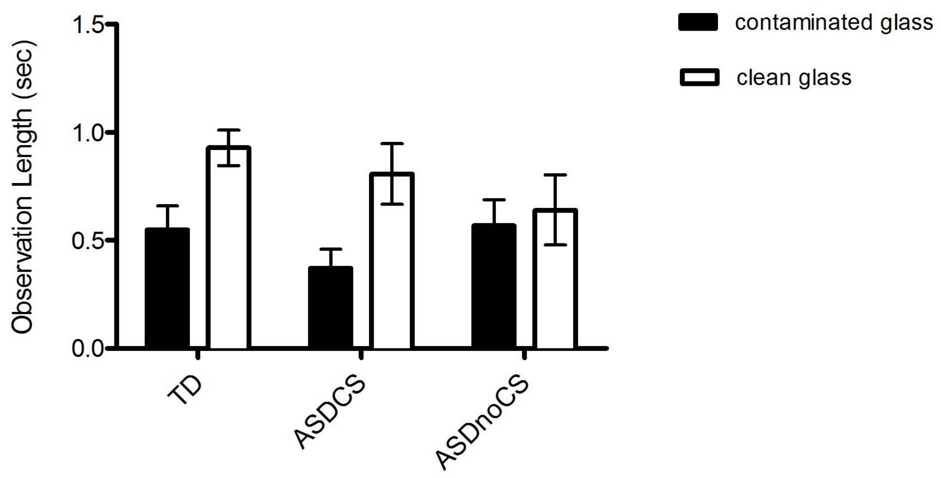


Figure 4.8: Observation length (sec) in the insect condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (contaminated *vs* clean). The contaminated glass was the glass containing the insect.

In the sugar condition, there was a significant main effect of glass , $F_{1,57}$=9.304, $p < .05$, , indicating that the participants discriminated between the two glasses. There was no a significant effect of interaction glasses x group $F_{2,57}$= 2.785, $p > .05$ (Figure 4.9).

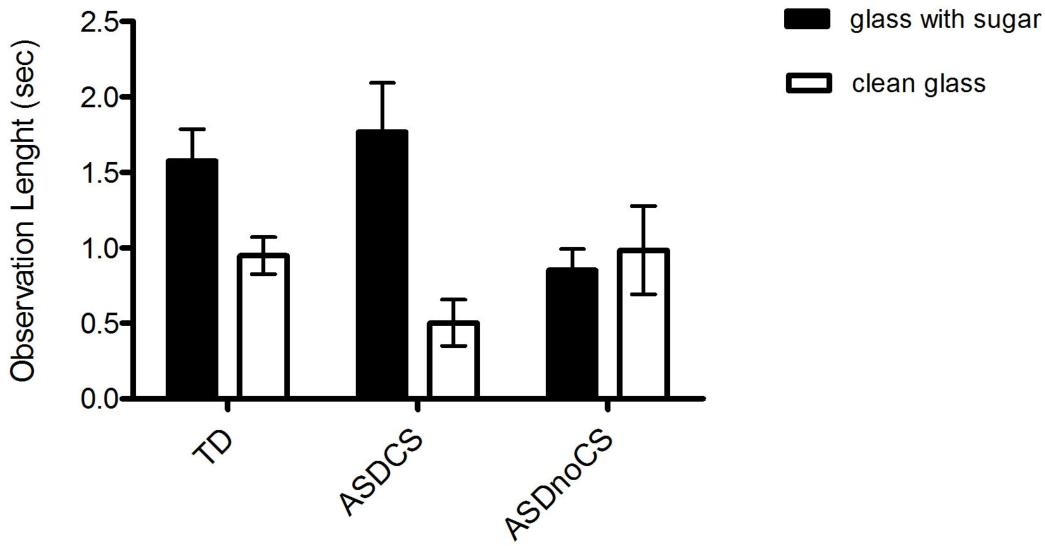


Figure 4.9: Observation Length in the sugar condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (glass with sugar *vs* glass with pure juice). The clean glass was the glass in which only pure juice was present.

We further investigate this phenomenon with a non-parametric analysis, by defining preferential looking in terms of delta: the looking time (observation length) to the correct gaze target (i.e. the uncontaminated glass in the contamination condition) minus the looking time to the incorrect gaze target (i.e. the contaminated glass in the contamination condition). A positive delta was considered to indicate a preference for the correct gaze target. Two children with ASD (one ASD CS and one ASD noCS) that did not show any preference (equal observation length toward the two gaze targets) in the insect condition were excluded from this analysis. Comparison of responses between the groups using one-tailed Fisher Exact Probability Tests showed that there was a significant difference on preferential looking between ASD CS and ASD noCS children (p=0.05). Children with ASD with contamination sensitivity were significantly more likely to look longer at the glass without the insect than ASD children without contamination sensitivity.

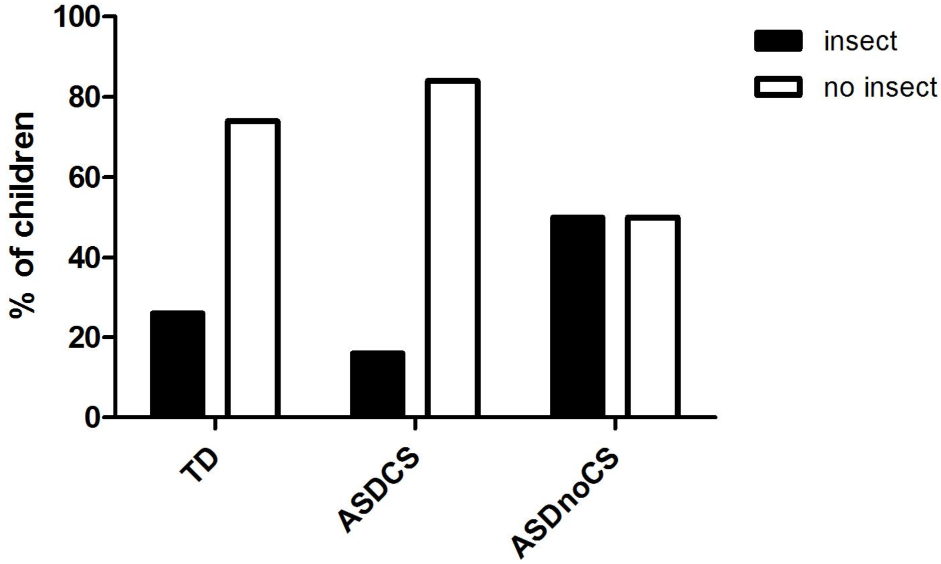


Figure 4.10: Percentage of children who showed preferential looking toward the glass with the insect or toward the clean glass in the insect condition. Preferential looking was evaluated by examining the difference (delta) between the Observation Length (sec) toward the contaminated glass (the glass in which an insect was floating on top of the juice) minus the Observation Length toward the uncontaminated juice. A positive delta indicates a preference toward the glass with the insect while a negative delta indicates a preference for the uncontaminated glass.

In the sugar condition (Figure 4.11), two children with ASD (one ASD CS and one ASD noCS) that did not show any preference (equal observation length toward the two gaze targets) were excluded from the non-parametric analysis of the looking preference. Fisher's Exact Probability Tests indicated that ASD noCS were not different from TD (p=0.3921), that ASD noCS were not different from ASD CS (p=0.3388) and that TD did not differ from ASD CS (p=0.5095).

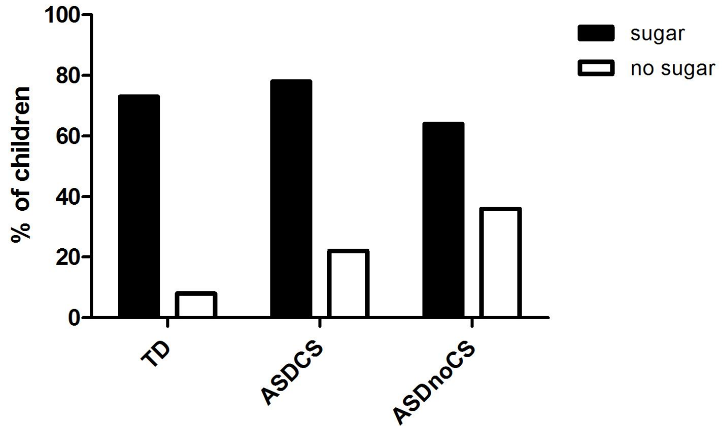


Figure 4.11: Percentage of children who showed preferential looking toward the glass with the sugar or toward the clean glass in the insect condition. Preferential looking was evaluated by examining the difference (delta) between the Observation Length (sec) toward the glass with the sugar minus the Observation Length toward the glass without sugar. A positive delta indicates a preference toward the glass with the sugar while a negative delta indicates a preference for the glass with pure juice.

Then we considered which one of the two glasses the children looked first. Two children (2 ASD CS) did not show any look first (equal latency of observation toward the two glasses) and therefore were excluded from the analysis of look first in the insect condition. Two children (1 ASD CS and 1 ASD noCS) did not show any preference and therefore were excluded from the analysis in the sugar condition. Fisher's Exact Probability Tests indicated that, in the insect condition, ASD noCS were not different from TD (p=0.4601), that ASD noCS were not different from ASD CS (p=0.5722) and that TD did not differ from ASD CS (p=0.3508) in which glass they looked at first.

In the sugar condition, the three groups did not differ in respect to which glass they looked first (X_2 (2, N =60) = 2.277, p >.05). The results are represented respectively in Figure 4.11 and in Figure 4.12.

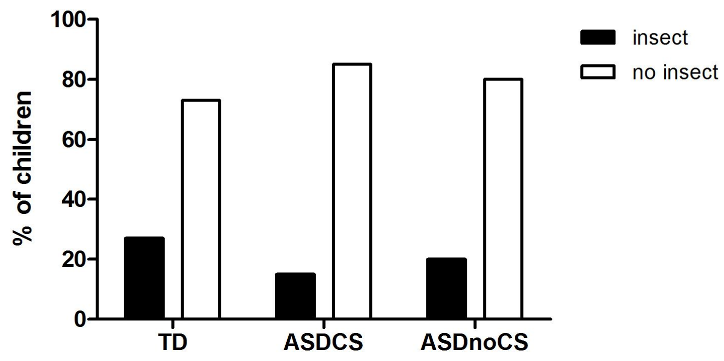


Figure 4.12: Percentage of children who looked first toward the glass with the insect or toward the clean glass with pure juice.

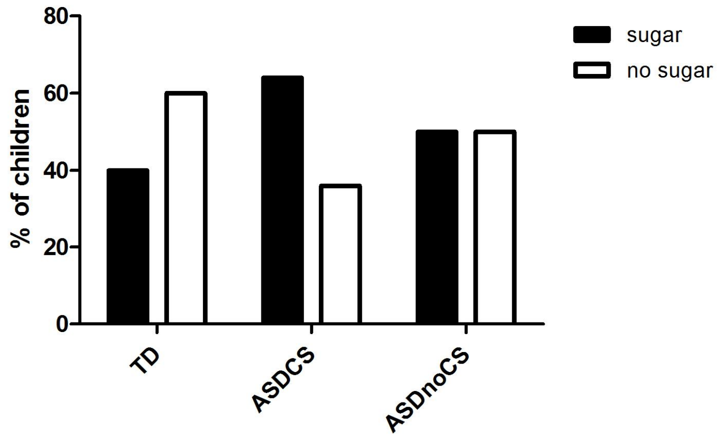


Figure 4.13: Percentage of children who looked first toward the glass with the sugar or toward the clean glass with pure juice.

When we analyzed the differences in the incidence of GI symptoms in relation to contamination sensitivity (Figure 4.13), there were no significant difference between the three groups ($X_2 (2, N = 40) = 2.147, p < .05$).

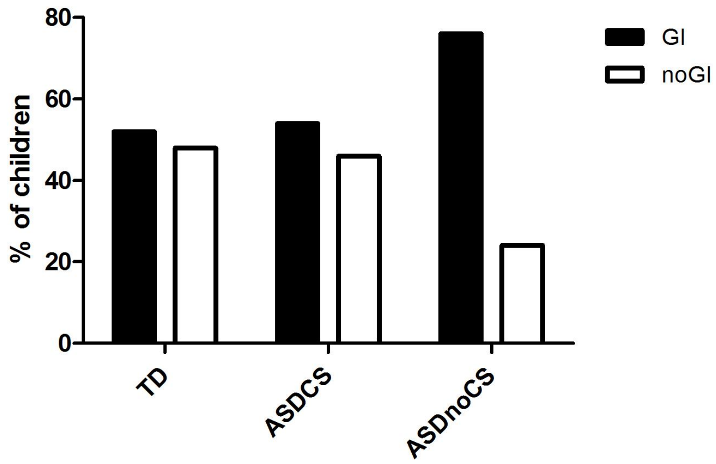


Figure 4.14: Percentage of TD, ASD CS, ASD noCS children with GI symptoms.

The three groups did not differ in the Disgust Sensitivity Scale Scores of the main caregiver, $F_{2,43}$=0.299; *p*>.05. A one-way ANOVA indicated significant differences between group in the Disgust Scores of the children as described by their caregiver, $F_{2,43}$ = 3.864; *p*<.05. A post-hoc test indicated that children with ASD who lacked contamination (*M*=11.55; *SE*=3.031) were significantly lower compared to TD (*M*=24.64; *SE*=1.684) and to ASD CS (*M*=24.40; *SE*=2.249).

4.6 RESULTS PART III: *estimation of pupil size*

To analyse the changes in pupil size under the effect of our stimulus, two time blocks were defined, anchored around the first fixation of the stimulus event (insect, sugar): baseline block and test block. The baseline block covered 250 msec prior to the stimulus event and the test block covered the following 250msec. In order to obtain a smooth timeline of pupil size, blinks were removed using the last valid pupil diameter before each blink as a replacement for the blink (Bernhardt et al., 1996). Changes in pupil size were estimated by a mixed ANOVA 2 (blocks) x 2 (conditions) x 3 (groups), which revealed a significant three way interaction block x condition x group ($F_{2,42}$ = 229,207 (p<0.05). There was an increase in attention from baseline to test in the insect condition (Figure 4.15) in TD and ASD CS children, while pupil size decreased in ASD noCS. In the sugar condition (Figure 4.16), there was an increase in attention from baseline to test in ASD noCS but not in TD children and ASD CS. A post hoc analysis revealed Post-hoc t-tests (Table 4.3) revealed that, while TD children and children with ASD CS tend to dilate their pupil size in response to the insect, children with ASD noCS tend to slightly restrict their pupil. In the sugar condition, ASD CS dilated their pupil, while TD children and ASD noCS children are quite stable.

condition	group	baseline	test	*t*(14)
INSECT	TD	3.821 (SD=0.007)	3.846 (SD=0.006)	-15.532*
	ASDCS	3.592 (SD=0.009)	3.613 (SD=0.007)	-8.152*
	ASDnoCS	3.604 (SD=0.009)	3.594 (SD=0.011)	-2.349*
SUGAR	TD	3.72 (SD=0.007)	3.725 (SD=0.005)	-2.168*
	ASDCS	3.616 (SD=0.007)	3.59 (SD=0.008)	9.539*
	ASDnoCS	2.968 (SD=0.013)	3.018 (SD=0.017)	-25.617*

* *significance at 0.005*

Table 4.3: Post-hoc t-test comparison between baseline and test in the insect and in the test condition for the three groups.

To further investigate pupil reactions, we statistically analysed variations in pupil size over time separately for each condition, insect and sugar (see respectively Figure 4.15 and Figure 4.16), by calculating a slope value for each participant's data by computing a linear regression on each participant's data separately. Then, by the means of a one way ANOVA, we analysed whether there were statistical difference between groups in the mean of the slopes of participants' curves. The results showed not significant differences between groups in the insect condition, $F_{2,57}=0.55$ ($p>0.05$).

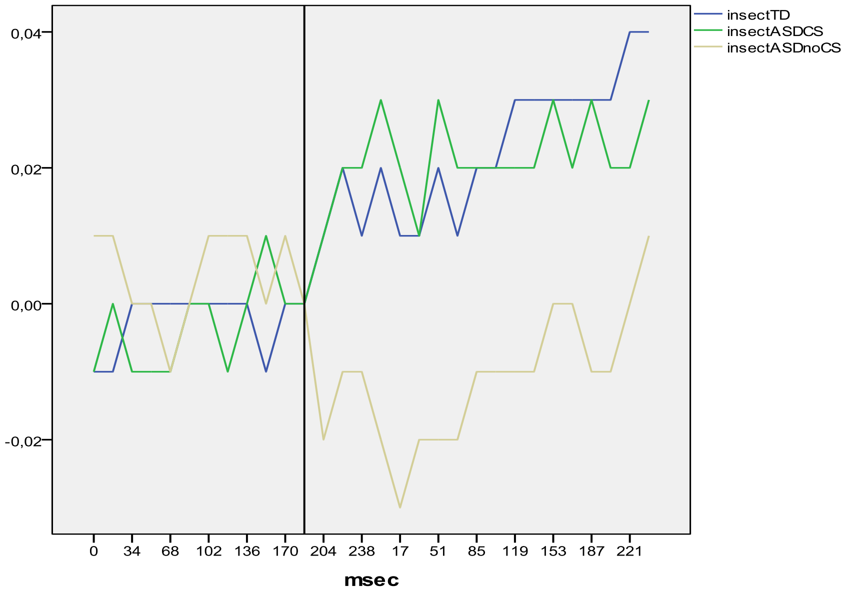


Figure 4.15: Time course of variations in pupil size in response to stimulus onset in the insect condition in TD children, children with ASD CS and ASD children without CS. The stimulus onset time is identified by a line which divides the baseline (before stimulus onset) and the test condition (after the stimulus onset).

There were not significant differences between groups in the sugar condition, $F_{2,57}$ =41,722 (p>0.05).

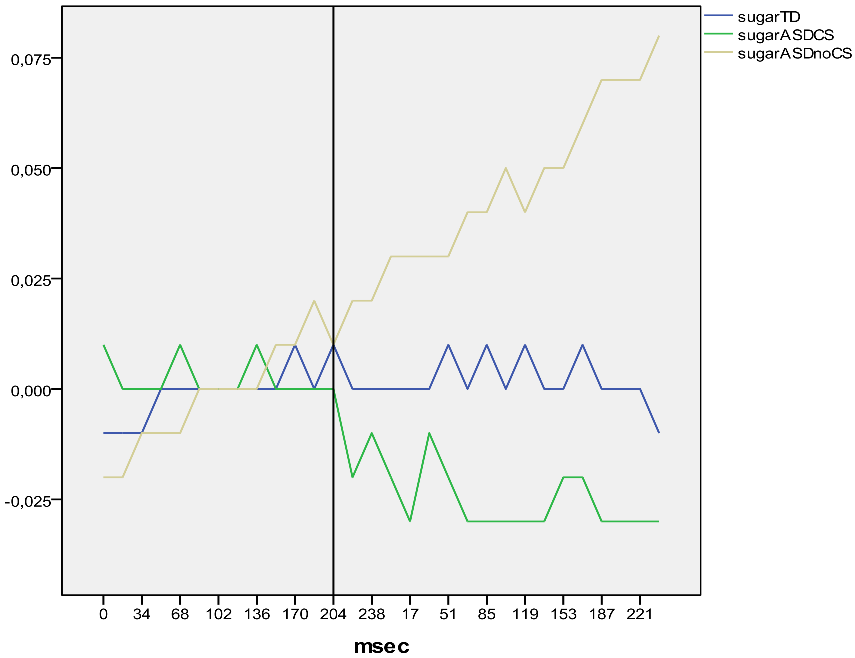


Figure 4.16: Time course of variations in pupil size in response to stimulus onset in the sugar condition in TD children, children with ASD CS and ASD children without CS. The stimulus onset time is identified by a line which divides the baseline (before stimulus onset) and the test condition (after the stimulus onset).

4.6 Discussion

This study aimed to investigate the implicit understanding of contamination in children with ASD compared with TD controls. First the children were evaluated for explicit contamination sensitivity, with the classical behavioural task (Siegal and Share, 1990). The results confirmed an high percentage of children with ASD lacking contamination sensitivity, as previously found in Study 1 and in Study 2. This result is in contrast with TD children, the totality of whom showed a strong sense of contamination.

Then, we selected two subgroups of children with ASD in relation to contamination sensitivity (ASD CS and ASD noCS0), matched for verbal, non-verbal and for chronological age, to investigate implicit contamination sensitivity by the means of preferential looking and variations in pupil size. The analysis of the looking behaviour in terms of number of fixation (FC) and in terms of length of fixation (OL) showed a general preference of all the participants in the three groups for one of the two glasses, indicating that the participants could discriminate between the two glasses and that they preferred one. However, no differences between groups were found and there were no effects of interaction glasses x group.

A non-parametric analysis of looking preference indicated significant differences in the looking preference of the two subgroups of ASD children: while ASD CS tended to “prefer” the glass without the insect, showing an implicit sense of contamination sensitivity, ASD noCS did not show any preference at all. However, when we analysed which of the two glasses the children looked at first, we did not found differences between the three groups in any of the two conditions (insect and sugar).

The investigation of contamination sensitivity by the means of variations in pupil size showed that children with ASD noCS didn’t show a pupillary response to the insect, while ASD CS and TD children dilated their pupils in response to the contaminant. In the sugar condition, ASD CS dilated in response to the sugar, while TD and ASD noCS didn’t show a sensible reaction.

Taken together, these results seem to indicate that children with ASD noCS don’t react to the insect, as if their learning process about the possible effects of external substances in a beverage might have been compromised not only at a semantic level but even at an implicit one. The results of ASD CS were mixed, since they showed a preferential looking for the uncontaminated glass but they dilated not only in response to the insect but also in response to the sugar. Their implicit response to contaminants is therefore different from ASD CS but also from TD children, that showed a preferential looking for the clean glass but that only dilated their pupil in response to the insect, as it was the most “interesting” substance in the juice (maybe the most unusual compared to the sugar). Considering these mixed results, more research are needed to better identify the most sensitive measure of implicit contamination sensitivity in ASD children.

ASD noCS children were judged lower in the Disgust Scores by their principal caregivers, as previously found in Study. Such a difference couldn’t be attributed to a different sense of disgust of the principal caregiver, since there were no differences in the Disgust Sensitivity Scale scores of the parents between the three groups. Thus, the parents in the three groups are equally likely to give to their children the appropriate information about food and contamination.

As in Study 2, children with ASD who lack contamination sensitivity did not differ in terms of GI symptoms compared to the other groups. Again, the profile of the subgroup of ASD noCS, even thought might seem at risk for unhealthy behaviours (no reactions to insects, both at explicit and at implicit level) is not characterized by an higher incidence of GI compared to the other ASD children and to TD controls.

Analysing the characteristics of the children with ASD who lack contamination sensitivity in comparison to the ones who show contamination sensitivity, we confirmed that the two groups did not differ in any other variable except for auditory processing abilities. These results highlight the importance of language and communication to promote a sense of disgust in very young children, especially in this clinical population. It might be of interest to investigate, in a subsequent study, if language could influence the reactions to disgust elicitors in a non-verbal contamination sensitivity task.

How language might enhance an intuitive understanding of contamination in children with ASD

5.1 Summary of the Chapter

In Study 2 and 3 we showed a deficit in auditory processing skills in children with ASD who lack contamination sensitivity, that might reduce the opportunities to learn from caregivers' messages about food. These results point to the beneficial effect of access to language on the development of contamination sensitivity. In this Chapter we further investigated this phenomenon, by implementing the same non-verbal paradigm used in Study 3, but this time incorporating an additional linguistic message about contamination. Specifically, we told the children that there is an insect in the juice and that the insect is disgusting and we observed their reactions in terms of preferential looking and variations in pupil size. The effect of linguistic information on implicit contamination sensitivity was evaluated in two subgroups of ASD children, that respectively lacked explicit contamination sensitivity (ASD noCS) and showed explicit contamination sensitivity (ASD CS) in a classical contamination task (Siegal and Share, 1990), and a group of TD controls. The two subgroups of children with ASD were selected by the means of a one-to-one matching procedure for verbal, non-verbal and chronological age. The results demonstrated an effect of language on implicit sensitivity to disgust elicitors in TD children and ASD children with contamination sensitivity, as measured by a classical contamination sensitivity task (Siegal and Share, 1990), but not in ASD children who lack contamination sensitivity measured by a classical contamination sensitivity task (Siegal and Share, 1990). As the two ASD groups were matched one-to-one for verbal, non-verbal and chronological age, our results highlight the importance of auditory processing skills for the development of contamination sensitivity.

5.2 Introduction

In Study 3, using an eye-tracking preferential looking paradigm, we explored to what extent children with ASD who lacked contamination sensitivity in a classical behavioral task were implicitly sensitive to disgust elicitors observed in a video. TD children and children with ASD who refused to drink a contaminated juice showed a strong looking preference for an uncontaminated drink, in sharp contrast to children with ASD who did not possess contamination sensitivity. As the two ASD groups did not differ in chronological or mental age but only in auditory processing skills, which were lower in children with ASD who lacked contamination sensitivity, our results point to the importance of auditory processing skills for the development of contamination sensitivity. However, the possibility that language might "enhance" contamination sensitivity was not explored.

As Rozin et al. extensively discussed in their classical papers (see for example: Rozin, Fallon, Augustoni-Ziskind, 1985; Rozin, Fallon, 1987), contamination might be learned from language in the course of daily conversations, in which adults teach children some basic assumptions about edible and inedible food. First, children are taught that contamination is determined by causally relevant factors, the contaminants, and hence it can be inferred from contextual information. For example, if an insect drops into the food it makes the food dirty by mere contact. Second, children are taught that a food that has been in contact with a contaminant is always in contact even though the contaminant has been removed. There are microscopic and "invisible" particles that continue to exist in the food even though the contaminant has been removed without a trace. It is through language and communication that children learn such basic concepts about contamination, during a continuous and generalized process of socialization in which they are helped to distinguish between edible and inedible substance.

Children at about 4 years of age already possess some understanding of these assumptions: they understand that contamination is causally determined by contact with a contaminant and that the food continues to be contaminated even though the contaminant will be removed. Moreover, their "attention" toward contaminants should be enhanced by linguistic information. On the contrary, children with ASD who lack contamination sensitivity, should be indifferent to any novel linguistic information about contaminated food due to their deficits in auditory processing skills.

On the basis of these considerations, we developed a study in which the effect of informative language on implicit contamination sensitivity was investigated, by telling children that there is an insect in the juice and that the insect is disgusting. We observed the reactions of the children in terms of preferential looking and variations in pupil size.

5.3 METHOD

Participants

30 TD children (15M; 15 F), aged between 3.9 years and 6.3 yrs (*M*=59.37; SD=7.649), and 37 children with ASD, aged between 3.10 years and 11.3 years (*M*=6.10 years; *SD*=24.714), diagnosed by expert clinicians on the basis of the DSM-IV criteria and the A.D.O.S. (Lord et al., 1999). Children with ASD were delayed in development and therefore they were older in chronological age compared to TD children. The TD children were recruited in public schools in Cagliari (Italy) while ASD children were recruited through referrals in a Centre for Pervasive Developmental Disorders in Cagliari (Italy). 15 of the children with ASD participated at the Study 3 (7 ASD CS and 8 ASD noCS)

Materials

The videos were presented with the Tobii T60 Eye Tracker. The tests for auditory processing were administered with a multimedia CD while the behavioural tasks were presented with pictures, small objects and toys.

Procedure

As in Study 3, the participants were first tested for explicit contamination sensitivity with the task developed by Siegal & Share (1990). In the explicit contamination sensitivity task (Siegal & Share, 1990), the experimenter offered to the child some juice, in the course of natural conversation during their snack time. Then, an insect float on top of the juice and the experimenter told to the child: “Here’s some juice. Oh! It has a cockroach floating on top”. The experimenter removed the insect without a trace and asked, “Is the juice OK or not OK to drink?”. The continued by telling the children a story in which the task will be to evaluate another’s responses to contamination.

The children with ASD with explicit contamination sensitivity (ASD CS) were matched one-to-one with the ASD children without contamination sensitivity (ASD noCS) for chronological age and for non-verbal and verbal mental-age, measured respectively by the Leiter International Performance Scale-Revised (Roid and Miller, 1997) and the Italian version of the Peabody Picture Vocabulary Test (Dunn & Dunn, 2000). The children that did not match for these three criterions were not included in the rest of the procedure. After few days, the two subgroups of ASD children (ASD CS and ASD noCS) and the TD controls were tested for implicit contamination sensitivity with an eye-tracking paradigm, involving the same set of videos developed for Study 3, implemented with linguistic information.

In the contamination condition (insect condition), the video showed an adult pouring some juice into two transparent glasses (the same quantity in each glass). Then, an insect floated on top of one glass of juice and the adult said: “How disgusting!!! There is a cockroach!” and removed it without a trace. Then, the adult actor asked to the child: “Which one is for you?”. The language accompanying the actions was meant to be highly informative, both emotionally and semantically, in order to stress the disgusting nature of the contaminant. In the purification condition, the adult poured some juice into two transparent glasses (the same quantity in each glass). Then, she put one spoonful of sugar into one of the glasses and she said: “How tasty!!! I put some sugar in here!” and poured in the sugar, stirred the juice and removed the empty spoon. Then, she asked to the child: “Which one is for you?”. The language accompanying the actions was meant to be highly informative, both emotionally and semantically, in order to stress the tasty properties of the sugar, which is meant to make the juice sweet and therefore nicer. The order of presentation of the two conditions was counterbalanced between subjects, as well as the position of the stimuli into the glasses (left *vs* right) and the direction of the grasping of the child in the baseline videos (left *vs* right).

To evaluate the possible role of individual differences in contamination sensitivity, children with ASD were tested for auditory processing with the LiP test (Archbold, 1994) and with a list of standardized non-sense words (Cornoldi, Miato, Molin, Poli, 1985). They were also evaluated for Theory of Mind abilities with tests of false and true beliefs (Woolfe, Want, & Siegal, 2002). The order of presentation of these tasks was counterbalanced between subjects.

Test for auditory processing: The Italian version of the Listening Progress Profile (LIP) developed by Sue Archbold (Nottingham Paediatric Cochlear Implant Program, 1994).

The Listening Progress Profile (LiP) measures the ability to detect, discriminate and identify sounds or voices. We administered five subtests: two subtests of detection of environmental sounds; a subtest of detection and identification; one subtest of detection of the human voice; one subtest of discrimination of the Ling sounds: AA, II, OO, SH, SS. The Ling sounds are characterized by audiometric properties whose spectrum is considered to be the basis of the development of human language. Moreover, we also administered a subtest of identification of two words. All the subtests have been administered with an interactive CD-ROM, which is meant to improve the motivation of the children to participate in the test thanks to a series of coloured and animated pictures and a number of visual and auditory rewards. Each subtest scored between 0 to 2, as follows:

- 0 = no sign of detection/discrimination/identification of any sound;
- 1 = sign of detection/discrimination/identification of some of the sounds;
- 2 = sign of detection/discrimination/identification of all the sounds.

The maximum score for all the subtest was 12, since one of the subtest was coded twice: once for detection and once for identification.

Standardized Nonsense Words Repetition task (Cornoldi, Miato, Molin, Poli, 1985).

The standardized NonsenseWords Repetition task (Cornoldi, Miato, Molin, Poli, 1985) comprises a list of non-words (sequences of letters that do not form words), characterized by growing levels of complexity. The sequences of letters that form the non-words in this test have been selected as having a high rate of occurrence in the Italian language. The task was scored by assigning one point for every syllable stated correctly. A wrong accent was scored as an error. The maximum score was 60.

The False Belief Task (Woolfe, Want, & Siegal, 2002).

The children are shown four ‘‘thought pictures’’: two in a false belief condition (FB), and the other two in a true belief condition (TB). The content of the pictures of FB and TB tasks was randomised across children. For each task, children were asked a belief and a reality question. They were scored as having passed the task if they answered both questions correctly. Each child therefore received a FB and a TB score from 0 to 4.

5.4 RESULTS PART I: *Explicit contamination sensitivity*

All the participants were evaluated with the classical contamination sensitivity task, developed by Siegal & Share (1990). The results (Fig. 5.1) showed that all the TD children refused to drink a contaminated juice, while only the 57% of ASD children showed contamination sensitivity (Fisher's exact test probability *$p<0.05$*).

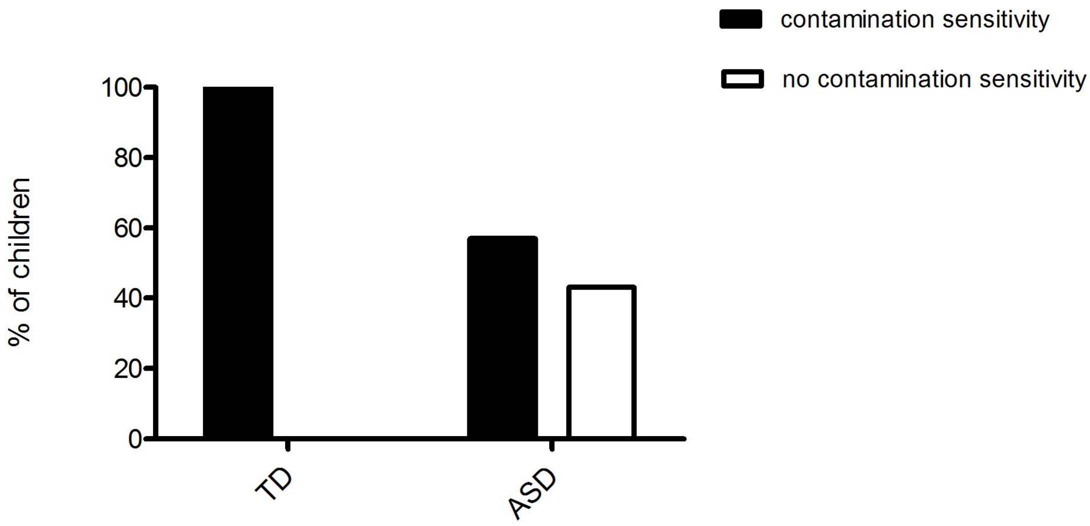


Figure 5.1: Percentage of Typically Developing children (TD) and children with ASD (Autism Spectrum Disorders) who refused to drink the contaminated juice (Contamination Sensitivity) and that agreed to drink the juice (No Contamination Sensitivity) in the classical contamination sensitivity task (Siegal and Share, 1990).

Following the explicit contamination sensitivity task, 15 children with ASD with explicit contamination sensitivity and 15 children with ASD without contamination sensitivity were matched one-to-one for chronological age, verbal and non-verbal mental age (Table 5.1). 5 participants were excluded after the matching because they did not match with any other participant and 2 participants were excluded because they received a diagnosis of a genetic syndrome.

	ASD CS n=15	*ASD noCS n=15*	*t* (28)	***p***
Chronological age Mean SE	86 mths 6.407	78 mths 6.383	0.936	p > .05
Nonverbal mental age Mean SE	86.53 6.824	81.92 5.570	0.506	p > .05
Verbal mental age Mean SE	70.23 4.268	63.20 6.166	0.909	p > .05

Table 5.1. Characteristics of children with ASD in regard to contamination sensitivity.

5.5 RESULTS PART II*: Looking Preference*

First we considered the number of Fixations (Fixation Count = FC) toward the two glasses in the three groups, separately for each condition (insect and sugar). A 2 (glasses: with and without insect) X 3 (group: TD, ASD CS and ASD noCS) mixed-model ANOVA revealed a significant main effect of glass, $F_{1,57} = 5.132$; $p < .05$, indicating that the children fixated more times at one of the two glasses. There was also a significant effect of interaction glass X group, $F_{1,57} = 5.132$; $p < .05$. A post-hoc comparison revealed that TD children fixated more times the glass with the insect (*M*=3,43; SD=1,906) compared to the glass without insect (*M*=1,37; SD=1,426), t(29)=4,852, $p<0.05$. There were not significant differences between the FC toward the two glasses for and ASD CS children and for ASD no CS (Figure 5.2).

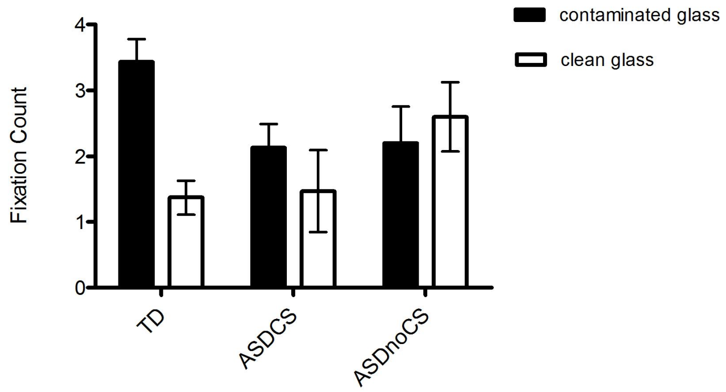


Figure 5.2: Fixation Count in the insect condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (contaminated *vs* clean). The contaminated glass was the glass containing the insect.

In the sugar condition, the mixed-model ANOVA revealed a significant main effect of glass, $F_{1,57} = 7.592$; $p < .05$, indicating that the children looked more times at one of the two glasses. There was not a significant effect of interaction glass X group, $F_{2,57} = 0.737$; $p > .05$ (Figure 5.3).

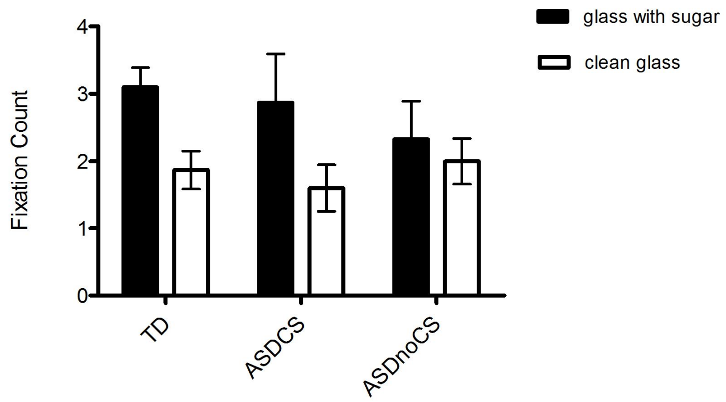


Figure 5.3: Fixation Count in the sugar condition - error bars represent standard errors - of Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (glass with the sugar vs glass with pure juice).

Then, we considered the length of observation (OL) toward the two glasses (with the insect or with the sugar), separately in each condition (insect/sugar). A 2 (glasses: with and without insect) X 3 (group: TD, ASD CS and ASD noCS) mixed-model ANOVA in the insect condition revealed a significant main effect of glass, $F_{1,57} = 5.132$; $p < .05$, indicating that the children looked more times at the glass with the insect. There was also a significant effect of interaction glass X group, $F_{1,57} = 5.132$; $p < .05$. A post-hoc comparison revealed that TD children observed longer the glass with the insect (*M*=1,1957; SD=0,725) compared to the glass without insect (*M*=0,4012; SD=0,423), t(29)=4,972, *p<0.05*. There were not significant differences between the OL toward the two glasses for and ASD CS children and for ASD no CS (Figure 5.4).

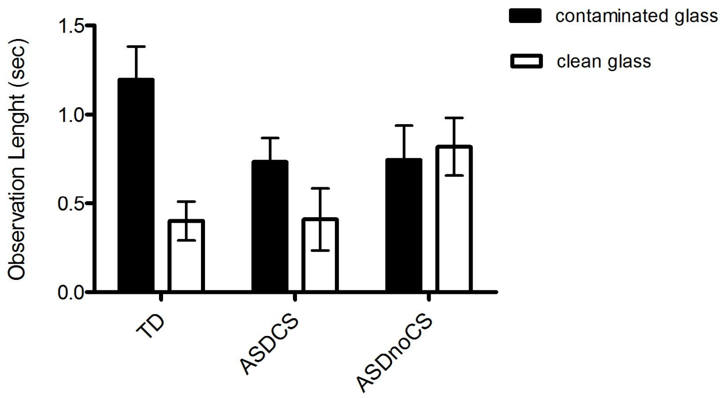


Figure 5.4: Observation length in the insect condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (contaminated vs clean). The contaminated glass was the glass containing the insect.

In the sugar condition, no significant effects were found (Figure 5.5).

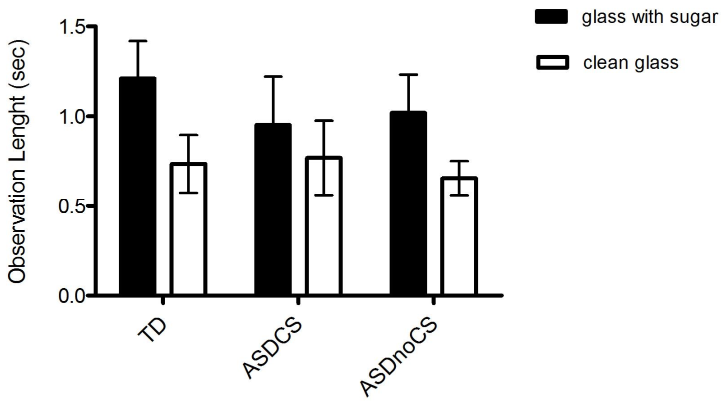


Figure 5.5: Observation length in the sugar condition - error bars represent standard errors - for Typically Developing Children (TD), children with ASD with contamination sensitivity (ASD CS) and children with ASD without contamination sensitivity (ASD noCS) toward the two glasses (glass with the sugar vs glass with pure juice).

We further analysed the looking preference with a non parametric analysis. Preferential looking was measured in terms of delta: the looking time (observation length) to the correct gaze target (i.e. the uncontaminated glass in the contamination condition) minus the looking time to the incorrect gaze target (i.e. the contaminated glass in the contamination condition). A positive delta was considered a preference for the correct gaze target. In the insect condition (Figure 5.6), two children (1 ASD CS and 1 ASD no CS) did not show any preference (equal observation length toward the two gaze targets) and therefore were excluded from the analysis in the insect condition. Comparison of responses between the groups using Fisher Exact Probability Tests indicated that ASD noCS were not different from TD (p=0.0184), that ASD noCS were not different from ASD CS (p=0.1730) and that TD did not differ from ASD CS (p=0.2980).

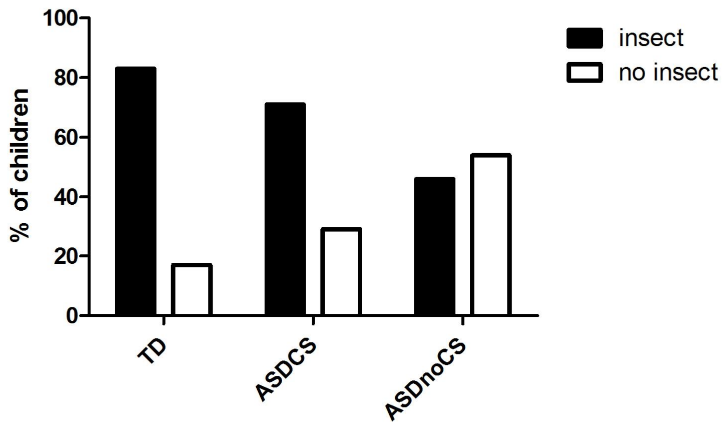


Figure 5.6: Percentage of children who showed preferential looking toward the glass with the insect or toward the clean glass in the insect condition. Preferential looking was evaluated by examining the difference (delta) between the Observation Length (sec) toward the contaminated glass (the glass in which an insect was floating on top of the juice) minus the Observation Length toward the uncontaminated juice. A positive delta indicates a preference toward the glass with the insect while a negative delta indicates a preference for the uncontaminated glass.

Two children (2 ASD no CS) did not show any preference (equal observation length toward the two gaze targets) and therefore were excluded from the analysis in the sugar condition (Figure 5.7). Comparison of responses between the groups using one-tailed Fisher Exact Probability Tests indicated that ASD noCS were not different from TD (p=0.4001), that ASD noCS were not different from ASD CS (p=0.3800) and that TD did not differ from ASD CS (p=0.5480).

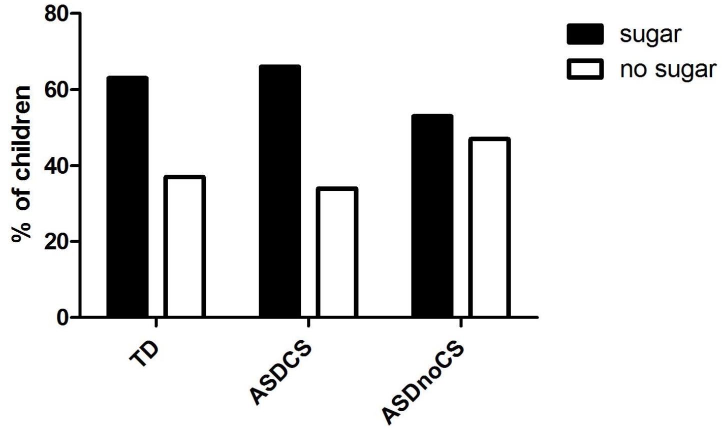


Figure 5.7: Percentage of children who showed preferential looking toward the glass with the sugar or toward the clean glass in the sugar condition. Preferential looking was evaluated by examining the difference (delta) between the Observation Length (sec) toward the glass containing the sugar minus the Observation Length toward the glass with pure juice. A positive delta indicates a preference toward the glass with the sugar while a negative delta indicates a preference for the glass with pure juice.

Two children (1 ASD CS and 1 ASD no CS) did not show any look first (equal latency of observation toward the two glasses) and therefore were excluded from the analysis of look first in the insect condition. Two children (1 TD and 1 ASD CS) did not show any preference and therefore were excluded from the analysis in the sugar condition.

In the insect condition (Figure 5.8), comparison of responses between the groups using one-tailed Fisher Exact Probability Tests indicated that ASD noCS were not different from TD (p=0.5189), that ASD noCS were not different from ASD CS (p=0.5) and that TD did not differ from ASD CS (p=0.3320). In the sugar condition (Figure 5.9), the three groups did not differ in regard to which glass they looked first (X_2 (2, N =60) = 5.048, p >.05).

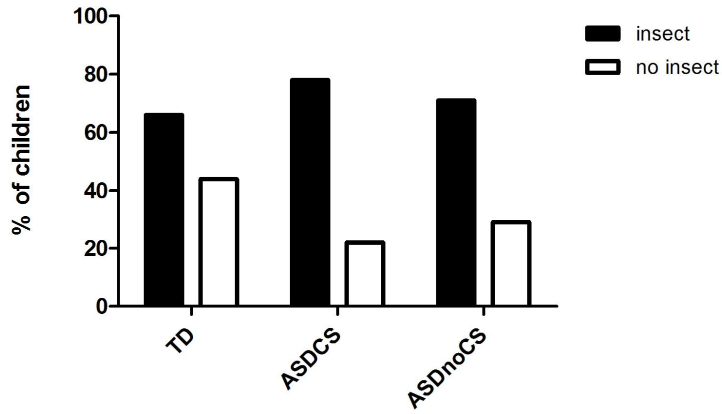


Figure 5.8: Percentage of children who looked first toward the glass with the insect or toward the clean glass in the insect condition.

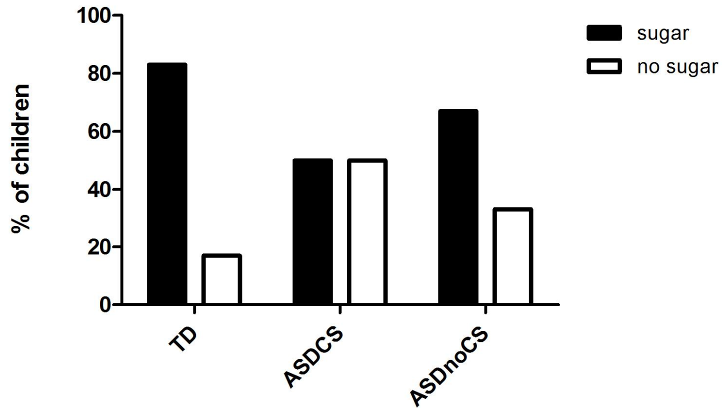


Figure 5.9: Percentage of children who showed a preferential looking toward the glass with the sugar or toward the clean glass in the sugar condition.

The three groups did not differ in the incidence of GI symptoms (X_2 (2, N =40) = 3.039, p <.05), shown in Figure 5.10.

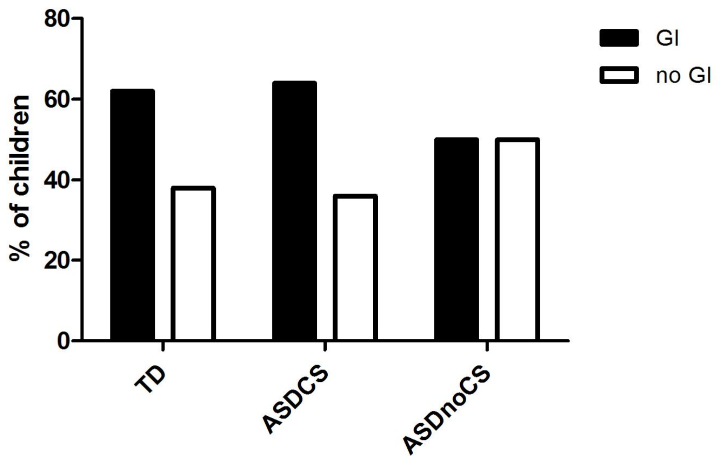


Figure 5.10: Percentage of TD, ASD CS, ASD noCS children with GI symptoms.

The three groups did not differ in the Disgust Sensitivity Scale Scores for the main caregiver (TD mean=58.26 (SE=2.668), ASD CS mean=64.50; (SE=4.872), ASDs noCS mean=62.64; (SE=3.217); $F_{2,43} = 0.935$; $p > .05$). Children with ASD who lacked contamination sensitivity were significantly lower in caregiver-estimated Disgust Scores compared to the children in the other two groups (TD mean=27.18 (SE=0.990), ASD CS mean=25.64; (SE=2.390), ASD noCS mean=19.27; (SE=2.450); $F_{2,43} = 5.053$; $p < .05$). To investigate the possible role of individual differences in contamination sensitivity, children with ASD in the two subgroups (with and without contamination sensitivity) were tested for auditory processing skills and Theory of Mind abilities. As shown in Table 5.2, the two subgroups significantly differ in auditory processing skills.

	ASD CS n=15	***ASD noCS n=15***	*t* (28)	***p***
Theory of Mind Mean SE	2.23 0.425	1.846 0.317	0.724	p > .05
Perception of sounds Mean SE	11.84 0.104	10.80 0.354	2.656	p < .05*
Non-words repetition Mean SE	48.46 2.040	40.14 2.313	2.681	p < .05*

Table 5.2. Individual differences in children with ASD in relation to contamination sensitivity

5.6 RESULTS PART III: *estimation of pupil size*

To analyse variations in pupil size under the effect of language, we applied the same procedure as Study 3. Two time blocks were defined, anchored around the first fixation of the stimulus event (insect or sugar): baseline block and test block. The baseline block covered 250 msec prior to the stimulus event and the test block covered the following 250msec. As suggested in Bernhardt et al. (1996), blinks were removed using the last valid pupil diameter before each blink as a replacement for the blink, to obtain a smooth timeline for pupil size. Changes in pupil size were estimated by a mixed ANOVA 2 (blocks) x 2 (conditions) x 3 (groups). There was a significant three way interaction block x condition x group ($F_{2,42} = 3.784$; $p < .05$). There was an increase in attention from baseline to test in the insect condition (Figure 5.11) in the three groups of children, even though the variation in the pupil size of ASD noCS children was smaller compared to the other groups.

Post-hoc t-tests (Table 5.3) revealed that, in the insect condition, the children of the three groups tend to dilate their pupil in response to the insect, event thought ASD noCS children dilated to a lesser extent compare to the other two groups. In the sugar condition, the children of the three groups tend to dilate their pupil in response to the insect, event thought ASD CS and ASD noCS children dilated to a lesser extent compare to the other two groups.

condition	group	baseline	test	*t*(14)
INSECT	TD	3.708 (SD=0.019)	3.792 (SD=0.027)	-27.465*
	ASDCS	3.295 (SD=0.024)	3.383 (SD=0.036)	-18.330*
	ASDnoCS	3.989 (SD=0.007)	4.025 (SD=0.021)	-7.407*
SUGAR	TD	3.74 (SD=0.016)	3.796 (SD=0.014)	-29.454*
	ASDCS	3.558 (SD=0.023)	3.581 (SD=0.014)	-2.554*
	ASDnoCS	4.014 (SD=0.007)	3.987 (SD=0.009)	7.678*

* *significance at 0.005*

Table 5.3. Post-hoc t-tests comparison between baseline and test in the insect and in the sugar condition for the three groups.

Then, we further analysed pupil reaction considering variations in pupil size over time in each condition: insect (Figure 5.11) and sugar (5.12). We calculated a slope value for each participant's data by the means of a linear regression on each participant's data separately. Then, we computed a one way ANOVA to analyse whether there were statistical difference between groups. The results showed not significant differences between groups in the insect condition, $F_{2,57}$ =0.070 (p>0.05).

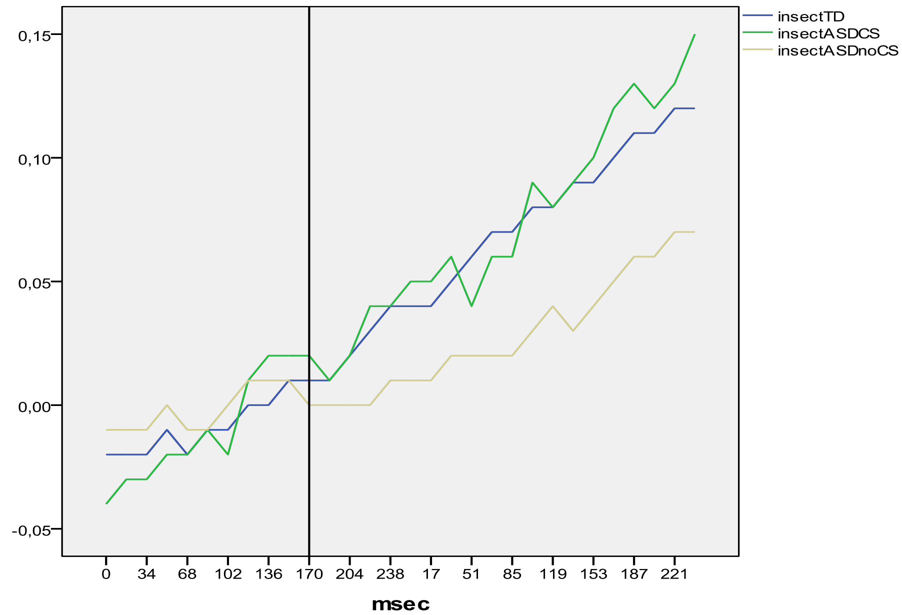


Figure 5.11: Time course of variations in pupil size in response to stimulus onset in the insect condition in TD children, ASD children with and without contamination sensitivity (respectively ASD CS and ASD noCS). The vertical line at 170 ms identifies the stimulus onset. This line divides the baseline (the period before the stimulus onset) from the test condition (after stimulus onset).

There were not significant differences between groups in the sugar condition, $F_{2,57}$ =0.279 (p>0.05).

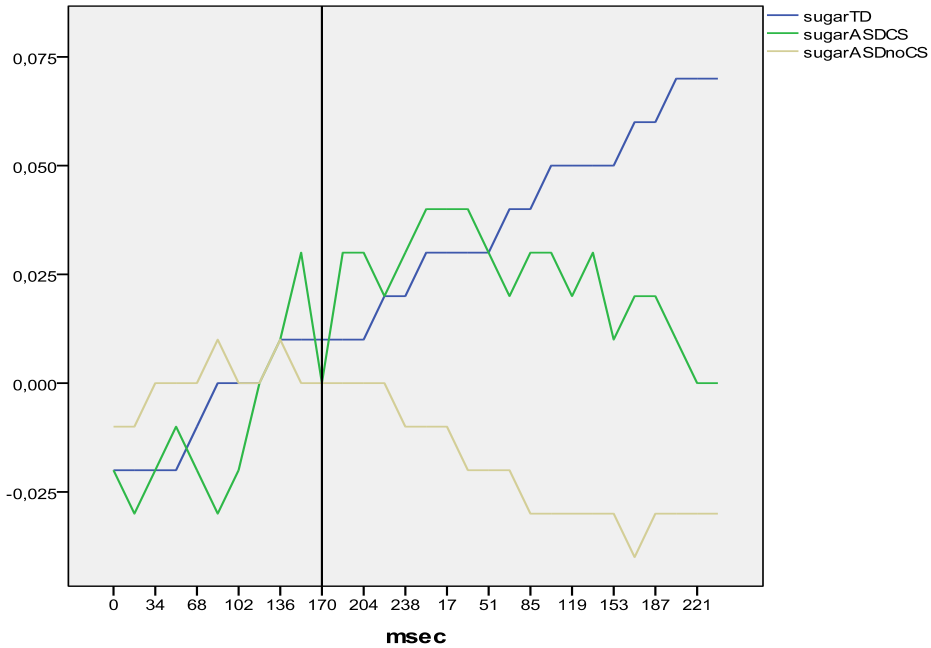


Figure 5.12: Time course of variations in pupil size in response to stimulus onset in the sugar condition in TD children, ASD children with and without contamination sensitivity (respectively ASD CS and ASD noCS). The vertical line at 170 ms identifies the stimulus onset. This line divides the baseline (the period before the stimulus onset) from the test condition (after stimulus onset).

5.7 Discussion

This study aimed to investigate the possible influence of language on the implicit contamination sensitivity in children with ASD compared with TD controls. As in Study 3, first the children were evaluated for explicit contamination sensitivity, with the classical behavioural task (Siegal and Share, 1990). The results confirm an high percentage of children with ASD lacking contamination sensitivity compared to TD controls, as previously found in Study 1, Study 2 and Study 3.

Then, we selected two subgroups of children with ASD in relation to contamination sensitivity (ASD CS and ASD noCS0), matched for verbal, non-verbal and for chronological age, to investigate implicit contamination sensitivity by the means of preferential looking and variations in pupil size. The parameters that defined the preferential looking were the number of fixations and the length of observation toward two glasses: one with an external glass (insect/sugar) and one without external substance.

The results showed that TD children fixated for more time and looked longer to the glass with the insect, like it was more “interesting”. This was not true for children with ASD, both with and without contamination sensitivity, who didn't show a statistically significant preference for one of the two glasses, triggered by the explicit linguistic information. Even though there was a tendency of ASD CS children to prefer the contaminated glass in the insect condition, this preference was not strong enough to be statistically significant. There were no differences between the three groups in the non parametric analysis of the looking preference, as well as in the timing of the first look to one of the two glasses. These results seem to indicate that the implicit perception of contaminants in ASD CS children is not a robust result: we found it in Study 3 but it was not confirmed in Study 4, even in the presence of an highly informative linguistic information. Thus, more studies are needed to better explore implicit contamination sensitivity in ASD children with eye-tracking techniques.

When we measured the effect of language in the variations of pupil size in response to a contaminant (the cockroach), the results showed that children with ASD who lacked contamination sensitivity showed an increase in attention to the insect, even though to a reduced extent compared to TD children and ASD children with contamination sensitivity. These results seem to indicate that these children display an interest through pupil dilation which might be enhanced by language information. It is not that they don't perceive language, it's just that they don't pay enough attention to its content, especially in the course of complex and unpredictable daily conversations.

In the case of our study, children were already focused on the stimuli so they were able to catch the language information and match it with the visual information, which enhanced their attention toward the insect. This effect, very subliminal and actually not really linked with a semantic level of understanding of hygiene and contamination, might be a window of opportunity for these children to gain new information about contaminants. It might be of interest to investigate, in a future study, the possible effect of a systematic training in contamination sensitivity in these children.

As in Study 3, children with ASD who lack contamination sensitivity were rated by their parents lower in disgust and contamination sensitivity. Such a difference cannot be attributed to a differences in the sense of disgust in the principal caregiver, since there are no differences in the Disgust Sensitivity Scale scores for the parents between the three groups. Moreover, children with ASD who lack contamination sensitivity did not differ in GI symptoms compared to the other groups. Again, the lack of explicit and implicit contamination sensitivity seems to don't be an actual risk factor to ingest contaminated substances and therefore developed an higher incidence of GI symptoms in ASD children.

Chapter 6

Summary, future directions of research, and conclusions

6.1 Summary of the Chapter

This Chapter summarizes the content of the thesis around three main topics: contamination sensitivity, cognitive abilities and auditory processing in ASD, implicit *vs* explicit contamination sensitivity in ASD and contamination sensitivity and GI symptoms in ASD. The results of the four studies are interpreted in the light of the principal theoretical background to the studies and previous empirical evidence illustrated in Chapter 1. Possible alternative explanations of the results are discussed, along with empirical evidence supporting our conclusions. Finally, some limitations of the studies are outlined along with future line of research, which might help to study more in depth the role of access to language on food rejection behaviour in children with ASD.

6.2 Contamination sensitivity, cognitive abilities and auditory processing

This thesis considers the general topic of disease-avoidant behaviour in children. Despite the possibility of treating and curing a number of acute and chronic diseases, individuals in Western societies are still vulnerable to a variety of infectious diseases. Thus, children need to learn very early on in development effective disease-avoidant behaviours to reduce the opportunities of infections. Specific cognitive abilities, which emerge during childhood, play an important role in the emergence of the biological concepts of disease (Siegal and Peterson, 1999). These concepts allow the individuals to recognize the perceptual cues that indicate the presence of a contaminant in the environment (Faulkher, Schaller, Park and Dunkan, 2004). However, the translation from “awareness” to “action” is not so automatic, but it seems to be mediated by a complex combination of cognitive, affective and socio-linguistic processes. Several cognitive abilities are responsible, like the distinction between appearance and reality (Siegal and Share, 1990), the concept of tiny, invisible particles that continue to exist in the food even though the contaminant has been removed (Toyama, 1999; Au et al., 1993), the distinction between bodily and mental reactions to contamination (Kalish,

1997) and the understanding of the nature of germs and contagion (Raman & Gelman, 2005).

Affective reactions, belonging mainly to the realm of disgust, trigger disease-avoidance behaviour. This thesis has been designed around the operational definition of disgust proposed by Rozin in the 1980s. According to Rozin, disgust is a basic emotion which prevents humans ingesting or touching harmful substances that have been contaminated by noxious micro-organisms (Rozin and Fallon, 1987).

Differently from other forms of food rejections, like distate or danger, which are motivated by sensory characteristics like taste or odour or by possible harmful consequences of their ingestion, disgust is the rejection of food on the basis of ideational grounds. The majority of substances perceived as disgusting are animal products or features that humans share with animals (Rozin, Haidt and McCauley, 2000). Since in the majority of cultures individuals are strongly motivated to be distinct from animals, disgust helps individuals to achieve the social and cultural value of being different from other animals (Rozin, Haidt and McCauley, 2000).

The ideational grounds of disgust are highlighted also by a special property of disgust elicitors, called "contamination", that is the power to render an otherwise edible food inedible by physical contact. Contamination can be explained by a law of "sympatetic magic", called "contagion": things which have been in contact with each other continue ever afterwards to act on each other (Frazer, 1959). Rozin et al. (1986) demonstrated empirically that the contagion law operates in the domain of disgust simply by dropping a dead, sterilized cockroach into a glass of juice and then removing it without a trace. In Western societies, insects are a "classical" disgust elicitor which are thought to render an edible food inedible by mere contact. Individuals find this juice much less desirable than a different type of juice, which had been in contact with a neutral object for the same period of time. This was an original and highly effective task, which has been used in a number of subsequent studies to evaluate contamination sensitivity in children (see for example Siegal, 1988; Siegal and Share, 1990; Au, Sidle and Rollins, 1993). In this thesis, we decided to use this task to evaluate contamination sensitivity empirically in all of the four studies, in combination with other tasks aimed to evaluate some cognitive abilities which are known to play an important role in the rejection of contaminated food: the concept of tiny, invisible particles that continue to exist and maintain their properties even after become invisible after dissolution (Au, Sidle and Rollins, 1993); Theory of Mind abilities (Siegal and Blades, 2003); representational abilities in general (Zaitchik, 1990). The use of the classical contamination task have been strategic in order to obtain results that could be compared with previous studies on contamination sensitivity in TD children.

Along with an operational definition of disgust, Rozin delineated also a developmental model of disgust and contamination sensitivity. According to this model, disgust evolves from distaste, an innate reaction to bitter substances, toward sophisticated and psychological forms during late childhood thanks to a process of enculturalization (Fallon, Rozin and Pliner, 1984). It is in the course of natural conversation that children are taught how to recognize and to react to contaminants, thanks to the mechanism of social referencing and through language and communication. This model has been supported by some studies showing that parents and other caretakers spend a considerable amount of time during the first two years of life trying to teach to the children what not to put into the mouth (Dunn, 1986; Toyama, 2000). Other studies have shown a strong resemblance between children and parents preferences about food (Rozin, Fallon, Mandel, 1984; Stevenson et al., 2010). Moreover, a cross-cultural study demonstrated significant differences between Hindu Indian and American children's responses to situations of potential contamination (Hejmadi, Rozin, & Siegal, 2004).

However, the majority of these studies were correlational, so the socio-cognitive hypothesis of contamination sensitivity development is still controversial and it needs to be further investigated. One possible way to assess how access to language and to family conversation affects the development of contamination sensitivity might be to observe food rejection behaviour in individuals naturally impoverished in language, like ASD. On the basis of these consideration, this thesis investigated how access to language influences contamination sensitivity in children, by investigating food rejection behaviours in children with ASD, who are known to be particularly impaired in attending to voices and speech (see for example Ceponiene et al., 2003; Kuhl, Coffey-Corina, Padden, & Dawson, 2005). If language plays a major role in this ability, ASD children should be at risk of not developing a sense of contamination during childhood.

Even though Rozin's model indicates the emergence of contamination sensitivity after 7 years of age (Fallon, Rozin and Pliner, 1984), a number of subsequent studies have found that even younger children, aged between 4 and 5, show some knowledge of contamination (Siegal and Share, 1990; Kalish, 1997; Springer and Belk, 1994; Toyama, 1999). Thus, we decided to investigate contamination sensitivity in preschool children. As described in Chapter 2, Study 1 replicated Kalyva et al. (2009) study, which is the only study so far that investigated contamination sensitivity in ASD children in comparison to TD pre-school children and children with DS. These three groups of children were particularly suitable to explore the role of language and communication in contamination sensitivity. While in TD children, intact social and cognitive abilities sustain contamination sensitivity at the age of four, in children with DS and in children with ASD there is an interesting dissociation between language and cognition that might exert an effect in the emergence of a sense of disgust, beside a broad developmental delay. Children with DS are characterized by social abilities which are often higher compared to their cognitive abilities. In contrast, children with ASD might preserve quite intact cognitive abilities despite low social competences. Therefore, any differences between these clinical population and typically developing children in contamination sensitivity might highlight the possible role of linguistic or cognitive abilities in the emergence of contamination sensitivity.

All the participants were tested for contamination sensitivity with a classical contamination task (Siegal & Share, 1990). The results of Kalyva et al. study (2009) demonstrated that contamination sensitivity is lacking in some children with ASD but not in the majority of TD children or children with learning disabilities, who refused to drink juice that had been in contact with an insect. Differently from Kalyva's study though, which employed some TD and children with DS from Greece and some TD and children with ASD from Italy, our study controlled for the possible effect of cultural differences by employing all the children from Italy.

The results of our Study 1 replicated Kalyva et al. (2009) findings, confirming a lack of contamination sensitivity in almost half of the children with ASD. Children with ASD and with DS were equivalent for non verbal and verbal mental age. Moreover, children with ASD without contamination sensitivity were equivalent to children with ASD that refused to drink the juice in a number of cognitive abilities, which are thought to be necessary for contamination sensitivity: Theory of Mind, non-mental representation and the concept of tiny, invisible particles that continue to exist in the food, even though the contaminant has been removed. This last ability is known to be necessary to understand the basic concepts about contamination (Au et al., 1993). Children with ASD who lacked contamination sensitivity were able to understand that a substance can continue to exist and maintain its inherent properties, even after it has become invisible. However, they didn't use this concept in deciding whether a food is edible or not after contamination.

Taken together, these results demonstrated that, despite intact cognitive abilities, some children with ASD lack contamination sensitivity at the same developmental age at which this ability is present in TD children. Because the three groups of children differed only in language and social impairments, Study 1 pointed to the key role of access to language in promoting contamination sensitivity in children.

As we described in Chapter 1, language and communication impairments in children with ASD are known to be grounded in auditory processing deficits, which lead to an abnormal perception of voices and speech at different levels of the nervous system: from the auditory cortex to the STS, the brainstem and the insula. The auditory abnormalities and consequent sensory deprivation might exacerbate the communication deficit in ASD, since successful communication relies on being able to both produce and process speech sounds in a meaningful manner. Consequently, if language plays a major role in the development of disgust, it is reasonable to predict that children with ASD who show higher auditory processing scores will be more aware of contamination than children with lower auditory skills. This hypothesis was tested in Study 2 (Chapter 3), in which we evaluated a group of ASD children and TD children for auditory skills in relation to contamination sensitivity, with the Italian version of the Listening Progress Profile (LiP) developed by Archbold (1994) and the Standardized Non-Word Repetition task (Cornoldi, Miato, Molin, Poli, 1985).

The LiP test measures a range of early listening skills (detection, discrimination, identification) from the first response to environmental sounds to the first response to voice. Moreover, the LiP test evaluates the processing of the so called “Ling sounds”, whose audiometric properties are considered to be the basis of the development of human language (Nikolopoulos, Wells & Archbold, 2000). The Standardized Non-Word Repetition task is a phonemic discrimination task that requires children to repeat non-words of 1-4 syllables, right after that they have been stated by the experiment one by one. Phonemic discrimination is crucial for language manipulation, which plays a fundamental role for language acquisition and oral language (Bishop, North, & Donlan, 1996). The results of Study 2 confirmed what was found in Study 1, that some children with ASD lack contamination sensitivity, in contrast with TD controls. A lack of contamination sensitivity in ASD was linked to a deficit in auditory processing abilities, while children with ASD who refused to drink a contaminated juice were characterized by the same auditory processing skills as controls.

The findings of Study 2 are in line with previous studies, showing that auditory processing deficits characterize some but not all individuals with ASD (Jones et al., 2009). Moreover, auditory deficit measures employed in Study 2 demonstrated that ASD children were characterized by a specific deficit in auditory processing of speech.

In the LiP test, developed to test environmental and human sounds (voices) perception in deaf children after a cochlear implants, none of the ASD children tested in Study 2 were absolutely "deaf". This is of particular interest, if we considered that ASD children are often initially misdiagnosed as deaf (Rapin, Katzman, 1998). These results indicate that it is not that ASD children cannot hear, it is that some of them tend to react less to environmental sounds and voices compared to controls. Even though we did not analyze individual differences in this task, it is important to highlight that two of the five subtest used employed human voices, which are known to be less effective than environmental sound to attract the attention of individuals with ASDs, despite intact abilities in perceiving the physical properties of the sounds (see for example Ceponiene et al., 2003; Whitehouse and Bishop, 2008; Kemner et al., 1995; Kuhl et al., 2000).

The ability to perceive spoken language was specifically assessed through the repetition of nonsense words. Lower abilities in this task indicate a difficulty in discriminating phonemes and an impoverished phonological working memory, or phonological loop (Baddeley, 1996), which is fundamental for auditory comprehension. This memory system temporarily stores novel phonological input, while other cognitive processes are involved in the attempt to understand the spoken language (Baddeley, Gathercole & Papagno, 1998). According to previous studies, ASD children should adequately perceive nonsense words due to their tendency to ignore the semantic level of spoken language (Jarvine-Pasley et al., 2008) and to an enhanced auditory perception of low-level perceptual information (Dakin & Frith, 2005; Happè e Frith, 2006; Simmons et al., 2009).

Contrary to this prediction, our results showed that some of the children with ASDs were lower in the repetition of nonsense words, both for simple (syllables) and complex (quadrisyllabic) items. These results confirm what was found in a previous study, indicating poorer speech discrimination for nonsense words in children with ASD (Heaton, Hudly et al., 2008). In Heaton's et al. (2008) study, pitch discrimination of sounds, monosyllabic real words and monosyllabic non-sense words was evaluated. Children with ASD showed enhanced pitch discrimination compared to controls. However, pitch discrimination was lower for speech like stimuli, both words and non-sense words. The use of non-words allowed them to conclude that it was speech, rather than the semantic content, that could be the cause of poorer speech discrimination.

As in Study 1, also in Study 2 there were no differences between the two subgroups of ASD children in several cognitive abilities which are crucial for contamination sensitivity: Theory of Mind, non-mental representation and the concept of tiny, invisible particles that continue to exist in the food, even though the contaminant has been removed. The two subgroups of ASD children were also equivalent for verbal and non-verbal mental age. Thus, these results indicate a detrimental effect of auditory processing deficits in contamination sensitivity in these children.

These findings turned to be quite robust, since they were replicated also in Study 3 and in Study 4, in the part of these studies in which we employed the same Experimental design of Study 2. The key role of auditory processing in contamination sensitivity was further supported in Study 4, in the part in which we exposed the participants to linguistic messages which pointed out that there was a cockroach in the juice and that this cockroach was disgusting. The reactions of the children were evaluated, in terms of preferential looking and variations in pupil size. The results showed that children with ASD who were lower in auditory processing ability (and lacked contamination sensitivity) were less sensitive to these messages compared to the other groups: their pupil dilated to a lesser extent compared to TD and ASD CS. It might be of interest to identify the minimum level of attention necessary to trigger explicit contamination sensitivity. These results of Study 4 are in line with what was found by Kuhl et al. (2005), who investigated for social orienting to speech in ASD in combination with the processing of linguistic information. The results of Kuhl's et al (2005) study showed an association between social and linguistic process of speech in ASD, leading to the conclusion that children with ASD who fail to orient to language might be impaired also in speech discrimination.

A possible alternative explanation for the differences in contamination sensitivity in children with ASD found in our Studies 2, 3 and 4, beside auditory processing deficits, derives from Rozin's model of disgust. According to this model, children learn from their parents how to react in front of a contaminant. Thus, a reduced sense of disgust in parents might account for possible difference in contamination sensitivity in children. To explore this hypothesis, in Study 2, 3 and 4 we analysed Disgust Scale scores for the principal caregivers in relation to contamination sensitivity in their children, as shown by food rejection behaviour in the classical contamination sensitivity task. There were no significant differences in the Disgust Sensitivity Scale scores for the parents between TD children, children with ASD with contamination sensitivity and ASD without contamination sensitivity. However, children with ASD who lacked contamination sensitivity were perceived as less sensitive to disgust and contamination by their caregivers than the other two groups, indicating that their lack of contamination sensitivity is translated into daily life as a lack of disease-avoidant behaviours compared to TD and ASD children who show contamination sensitivity.

6.3 Implicit vs Explicit contamination sensitivity in children with ASD

In Study 1 and in Study 2, we found that many children with ASD lack contamination sensitivity in a classical contamination task. Classical contamination sensitivity tasks (Siegal and Share, 1990) evaluate behavioural reactions to contaminants by asking children if it’s OK to drink a juice that has been contaminated by an insect. This task is meant to be behavioural, in which the behaviour of the child tells us something about his/her understanding of contamination sensitivity: if a child refuses to drink the juice, it means that he/she understands the contaminating nature of the insect.

While this task has been widely used in typical populations, it has been used only in one recent study (Kalyva et al., 2009) with children with ASD, so its sensitivity for atypical children has yet to be established. It is possible that those children with ASD who were willing to drink the contaminated juice in our Experiments actually had an implicit sense of disgust and contamination but simply they misbehaved in the classical contamination task.

In order to be successful in a behavioural task, a child needs to communicate his/her choice to the Experimenter, either verbally or non verbally. Since children with ASD are known to be particularly impaired in social communications, both at verbal and non-verbal level (Tager-Flusberg, 1996), they might fail to communicate their choice to the Experimenter even though the children have some kind of awareness of contamination.

Moreover, to solve the classical contamination task, the child needs to inhibit his/her impulse to drink a liked drink (the juice offered by the Experimenter) even though he/she knows that the drink is not drinkable. Since children with ASD are known to be characterized by some deficits in executive functioning, like the lack of inhibition of a prepotent response (Hill, 2004), they might fail to show food rejection behaviours because of this.

On the basis of these considerations, we wondered whether ASD children that lack contamination sensitivity in the behavioural task actually didn't feel disgusted at all observing an insect floating on top of the juice or whether they could not communicate their feeling of disgust or whether they could not refrain from drinking the juice.

With these questions in mind, we designed two more studies, Study 3 and Study 4, in which we developed a non verbal experimental paradigm to test an implicit reaction to contaminants. The idea was that a non verbal task might provide a more sensitive measure of contamination sensitivity, because it does not require either a verbal judgement nor a voluntary choice between two alternatives.

Two measures that were meant to correct, respectively, for socio-communication and executive functioning deficits in children with ASD were employed: preferential looking and variation of pupil size. The first measure was obtained by tracking visual attention as index of "preference" toward one of two identical glasses of juice: one glass had been contaminated with an insect while the other glass contained uncontaminated juice. The second measure was the variation in pupil size, which is an involuntary, unconscious response to a contaminant, controlled by sub-cortical components of the nervous system (Wilhelm, Wilhelm and Ludtke, 1999). Thus, variations in pupil size are completely independent by the frontal cortex and therefore not related to executive functioning.

The combination of these two measures might account for implicit contamination sensitivity in children with ASD, beyond their lack in language and communication abilities and their deficits in executive functioning.

In Study 3 (Chapter 4) we explored to what extent children with ASD who lack contamination sensitivity in behavioural tasks are implicitly sensitive to disgust elicitors, through measures of looking time and variations in pupil size in response to a contaminant. A non-parametric analysis of looking preference indicated significant differences in the looking preference of the two subgroups of ASD children: while ASD CS tended to prefer the glass without the insect, showing an implicit sense of contamination sensitivity, ASD noCS did not show any preference at all. These results suggest that ASD children with contamination sensitivity can discriminate between the contaminated vs the uncontaminated juice, by looking longer on average at the uncontaminated juice. Thus, we can assume that the children "*know*" the difference between the two glasses, which appear identical but that are different because one has been contaminated by an insect. Such implicit knowledge was not present in children with ASDs who lacked contamination sensitivity.

In Study 3, TD children and children with ASD CS also showed significant increase in pupil size when observing a contaminant. This reaction was not found in ASD children who lacked contamination sensitivity. A variation in pupil size might indicate an unconscious perception of disgust elicitors, which precede the semantic interpretation of the stimuli. The lack of a pupillary response to contaminants in children with ASD who did not show contamination sensitivity in a behavioural task seems to indicate that their process of learning about contaminants has been impaired not only at a semantic and explicit level but also at the implicit one. Therefore, the insect is not a meaningful stimulus and doesn't elicit any emotional response at all. This lack of reaction might be due to the incapability of these children to show implicit emotional responses throughout pupil dilation. Previous studies indicated that usually pupil dilates in response to rewards in decision-making tasks (Bijleveld, Custer and Aarts, 200) and to pictures of desirable items (Laeng and Falkenberg, 2007). Indeed, ASD noCS did not show a dilation in pupil size in response to a spoon of sugar dropped into the juice, indicating that any external substance in the drink seems meaningfulness to them.

Variations in pupil size might be interpreted also in terms of changes in attention. Very recently, neuroscientists identified a strong link between pupillary response and the activation of the locus coeruleus and noradrenergic system (Koss, 1986). One current hypothesis is that the noradrenergic system, mediates the functional integration of the brain's attentional brain (Corbetta, Patel, & Shulman, 2008; Coull, Büchel, Friston, & Frith, 1999; Sara, 2009). The connection between pupil diameter, the locus coeruleus and the noradrenergic system allow researchers to measure pupil diameter in order to evaluate changes in attention, in a preconscious active state or when a conscious state cannot be made explicit either via verbal or motor responses (e.g., in preverbal infants, animals, and in some neurological patients). In the light of this model, therefore, pupil reactions found in our Experiment 3, both in TD and ASD children with contamination sensitivity might indicate that the insect elicited changes in the attentional system, which in turn prepared the cortex for further semantic elaboration of the stimuli. The lack of change in attention in ASD children without contamination sensitivity might be a sign that the insect was not recognized implicitly as an "interesting" stimulus, maybe due to a lack of previous experience in which the salience of the insect had been pointed out.

Preferential looking and variations in pupil size were employed also in Study 4, in combination with a classical behavioural task. In this study, we evaluated whether informative language about contaminants (telling the child that there is an insect in the glass and that the insect is disgusting) might influence implicit contamination sensitivity in children. The results showed that TD children fixated for more times and looked longer to the glass with the insect, like it was more "interesting". This was not true for children with ASD, both with and without contamination sensitivity, who didn't show a statistically significant preference for one of the two glasses, triggered by the explicit linguistic information. Even though there was a tendency of ASD CS children to prefer the contaminated glass in the insect condition, this preference was not strong enough to be statistically significant. These results seems to indicate that the implicit perception of contaminants in ASD CS children as measured in terms of preferential looking is not a robust result (we found it in Study 3 but it was not confirmed in Study 4), even in the presence of an highly informative linguistic information. Thus, more studies are needed to better explore implicit contamination sensitivity with eye-tracking techniques.

When we analysed variations in pupil size, in this study also children with ASD who lacked contamination sensitivity showed a dilation in pupil size in response to an insect, although to a lesser extent than the other two groups. This result indicates that, even though the visual information might be not relevant for ASD noCS, language was able to trigger attention, and hence it might represent a window of opportunity to teach to these children new knowledge about disgust and contamination. It might be of interest to investigate the minimum level of activation of attention which might be necessary to trigger explicit contamination sensitivity in these children.

In summary, the use of a non verbal paradigm in Study 3 and 4 lead to new research to explore biological knowledge in children with ASD, beyond their deficits in language and communication and impairments in executive control.

However, a possible alternative explanation for the differences in papillary responses between the groups might be that the attentional system functions in a different way in TD children and in children with ASD CS and with ASD noCS. To explore this hypothesis, we evaluated pupil dilation in the resting state. The resting state is a condition in which no visual stimuli were presented, but only a black screen was observed. Therefore, attention was not completely “off” but it was just not directed toward any visual stimulus. This is a rather extreme condition in which any variation to pupil size could be explained in terms of a basic way of functioning of the attentive system, as reflected in the pupil size.

We were able to test in a very preliminary way this hypothesis, by considering the variations of pupil size during the resting state of 20 TD, 10 ASD CS and 8 ASD noCS recorded with the eye tracker in study 3. We could not analyse the data of 10 TD, 5 ASD CS and 7 ASD no CS because they did not look sufficiently to the eye-tracker when the screen was black.

Very preliminary plots of the curves, representing the time course of the variations in pupil size during the resting state, showed that ASD children with contamination sensitivity (Fig. 6.1) and without contamination sensitivity (Fig. 6.2) showed a trend that was qualitative similar. Both of these trends looked markedly different from TD children (Fig. 6.3).

These results seem to indicate that any differences that we found between children with ASD CS and with ASD noCS in response to contaminants could not be ascribed to differences in the basic functioning of the attentive system during the resting state. However, this is only a qualitative analysis at the moment so any conclusion need to be considered with caution.

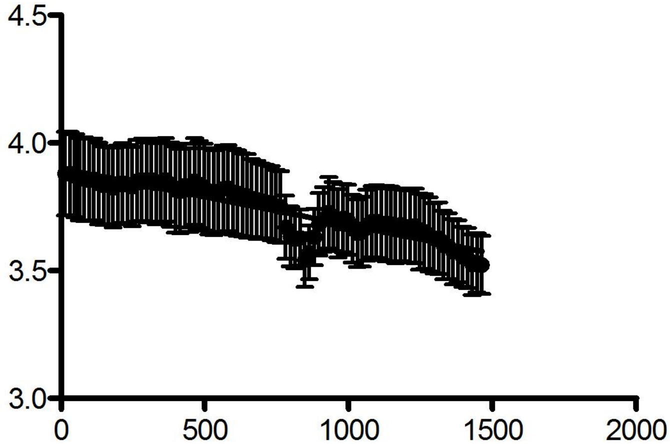


Fig. 6.1 Time course of variations in pupil size in resting state in ASD children without contamination sensitivity. The horizontal axis shows the time in msec. The vertical axis shows the size of pupil in mm

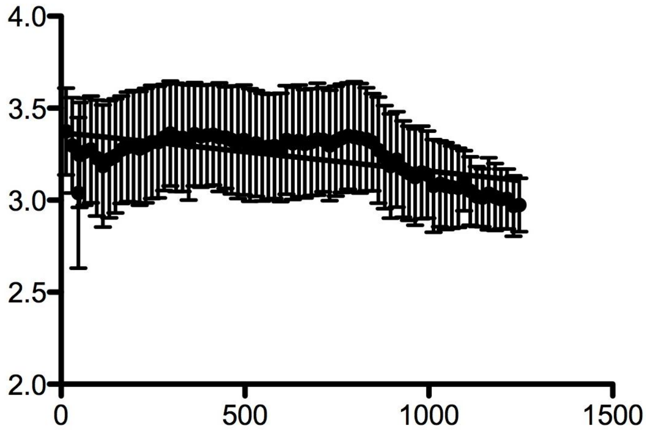


Fig. 6.2 Time course of variations in pupil size in resting state in ASD children with contamination sensitivity. The horizontal axis shows the time in msec. The vertical axis shows the size of pupil in mm

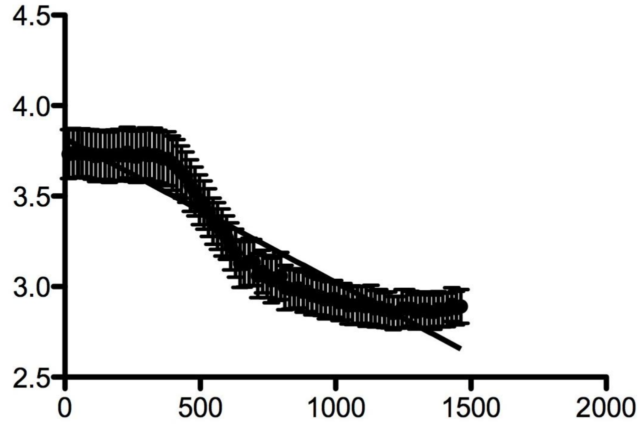


Fig. 6.3 Time course of variations in pupil size in resting state in TD children. The horizontal axis shows the time in msec. The vertical axis shows the size of pupil in mm

These differences will be the object of future analysis, in which we will consider more participants (from study 4) and we will evaluate statistical differences in the slope of the curves. If the qualitative differences that we plotted so far will be, our plan is to further investigate variations in pupil size in the resting state as a possible biomarker of ASD.

6.4 Contamination sensitivity and GI problems in ASD

As we described in Chapter 1, there is a protracted debate over the role of GI symptoms in ASD symptomatology. Some retrospective studies found a relatively low prevalence in ASD, similar to the general population (Fombonne & Chakrabarti, 2001; Taylor, 2002; Malloy et al., 2003). In contrast, other studies found GI symptoms in the 76% of the autistic population. Similarly, Valicenti-McDermott (2006) found that 70% of ASD children had GI problems, compared with 42% of the children with other neurological conditions and 28% of the children without neurological impairment. Very recently, Nikolov et al. (2009) found a prevalence rate of 22.7% for GI symptoms in a sample of 170 children with ASD. The prevalence of GI problems in ASDs, therefore, varies largely from study to study, probably due to a number of differences in the group evaluated and in the operational definition of GI symptoms (Buie, 2011). However, besides the possibly high prevalence of GI symptoms in ASD, for the parents of children with ASD the treatment and the prevention of these disorders is of great interest. GI problems tend to exacerbate behavioural problems in these children, like aggression and irritability, and there are some claims that gut inflammation might determine autistic symptoms (Wakefield, Murch, Anthony, Linnell, Casson, et al., 1998).

For these reasons, in this thesis we decided to investigate food rejection behaviour in children with ASD as a possible protective factor for GI problems in this population. In Study 2, 3 and 4, parents were administered a short questionnaire about GI problems in their children. Moreover, their perception of disgust and contamination sensitivity in their children was evaluated. To control for possible differences in teaching about disgust, parents Disgust Senstivity Scale scores were collected by administering to parents the DS-R (Olatunji, Williams, Tolin, Sawchuck et al., 2007). The results showed that, in each of our studies, children with ASDs who lacked contamination sensitivity did not differ from TD and children with ASDs with contamination sensitivity in the prevalence of GI symptoms.

However, we wondered whether such a lack of difference could be due to the size of the samples, which ranged from 23 to 30 in our studies for ASD children and from 27 to 30 for TD children.

To explore this possibility, we collapsed the participants of Study 2, 3 and 4, so we could evaluate the prevalence of GI symptoms in 141 children: 90 TD and 51 ASD. As shown in Table 6.1, ASD children did not differ significantly in GI symptoms in comparison to TD controls (X_2 (1, N =141) = 1.409, p >.05).

	GI symptoms	No GI symptoms
TD (n=90)	49 (54%)	41 (46%)
ASD (n=51)	33 (64%)	18 (36%)

Table 6.1: Percentage of TD and ASD children with Gastrointestinal symptoms.

Children with ASD (M=19.34; SE=8.96) were significantly lower in Disgust Scores as perceived by their parents, in comparison to TD children (M=24.25; SE=7.44), ASD, t(139)=3.390; p < .05). In relation to the Disgust Sensitivity Scale Scores as perceived by the main caregiver, ASD children (M=62.80; SE=13.09) did not differ compared to TD children (M=58.81; SE=13.76), t (139)= -1.614; p > .05).

Then, we considered GI symptoms in relation to contamination sensitivity, dividing the 141 participants of Experiment 2, 3 and 4 into the following subgroups: 90 TD, 26 children with ASD with contamination sensitivity and 25 children with ASD without contamination sensitivity. As shown in Table 6.2, the three groups of children did not differ significantly in the prevalence of GI symptoms (X_2 (1, N =141) = 1.409, p >.05).

	GI symptoms	No GI symptoms
TD (n=90)	49 (54%)	41 (46%)
ASD CS (n=26)	17 (65%)	9 (35%)
ASD noCS (n=25)	16 (64%)	9 (36%)

Table 6.2: Percentage of children with TD, ASD CS and ASD noCS with Gastrointestinal symptoms

Children with ASDs noCS (*M*=15.14; SD=9.117) who lacked contamination sensitivity were significantly lower in Disgust Scores compared to TD children (*M*=24.25; *SD*=7.441) and to ASD CS (*M*=22.73; SD=7.389), $F_{2,43}$ = 11.816; p < .05).

Children with ASDs noCS (*M*=63.10; SD=12.522) who lacked contamination sensitivity were significantly lower the Disgust Sensitivity Scale Scores as perceived by the main caregiver compared to TD children (*M*=58.81; *SD*=13.760) and to ASD CS (*M*=62.56; SD=13.814), $F_{2,43}$ = 1.302; p > .05).

Taken together, these results seem to indicate that children with ASD are not at greater risk of GI symptoms compared to TD children. Moreover, the lack of contamination sensitivity is not a risk factor, even though children with ASD who are not aware of the contaminants are rated by their parents as less competent in disgust and contamination compared to TD and to ASD children that show contamination sensitivity. Even though such a lack of knowledge is not translated into an actual higher prevalence rates for GI symptoms, these children need to learn more about biological concepts in order to adopt disease-avoidance behaviours in daily life in line with the children at the same age.

6.5 Limitations of the studies in this Thesis

Our studies were limited since with didn't consider other possible mediators of access to language and conversation about food and contamination in daily life. Even though children with ASD acquire a rich vocabulary, they are severely impaired in the ability to “map” the meaning of the words and the sentences by using a number of social cues present in the environment. A number of studies demonstrate that children with ASDs are often unable to follow the direction of the speaker's gaze (Baron-Cohen, Baldwin, & Crowson, 1997). The "core" of their deficit is the ability to use a combination of social cues, like eye-gaze, gestures and facial expressions that signal the speaker's intentions in the course of natural social interactions (Parish-Morris et al., 2007; Lord et al., 1999). Moreover, children with ASD are severely impaired in the use of language to engage in a reciprocal exchange of thoughts or experiences (see for example Wetherby, Watt, Morgan & Shumway, 2007). These difficulties emerge quite early in their childhood but persist throughout the lifespan (Dobbinson, Perkins, & Boucher, 1998). Each of them, paired with auditory processing skills, need to be considered as a potential risk factor that might interfere in the process of enculturalization, necessary for the development of contamination sensitivity.

6.6 Future lines of research

Future directions for research could include the possibility of investigating disgust in pre-verbal infants, in which language has still to emerge so it couldn't play any role yet in the emergence of disgust and contamination. These children should show, at around 2 years of age, a kind of implicit sense of contamination thanks to a process of socialization from parents and other care-givers, in the course of natural conversation. However, such knowledge should be very basic, extremely implicit, and it should constitute the framework for further development.

Another possible line of research might be to explore disgust sensitivity in deaf children, whose auditory impairments prevent them from focusing on daily communication about food and contamination. If language plays a major role in the emergence of contamination sensitivity, they should be quite similar to children with ASD in regard to contamination sensitivity.

A further direction might be to investigate whether a sense of disgust and contamination continue to exist in a population in which language has been active for a long time but it has been abruptly destroyed by physical damage, like in adults with aphasia. In these adults, disgust should continue to be present despite a lack of language and communication, because the individual has had the possibility of developing and consolidating this ability in the course of her/his prior life before the stroke.

Finally, it might of interest to pair the evaluation of contamination sensitivity in children with ASD with ERP measures. ERPs are a widely used technique to evaluate brain functioning in patients with cognitive diseases, by measuring electrophysiological responses of the brain to specific sensory, cognitive or motor event. This line of research might allow us to understand if and to what extent the brain reacts to visual and auditory information about disgust and contamination, in children impoverished in language development like children with ASD.

Last but not least, it might be of interest to develop an intervention study in which possible strategies to implement both the implicit and the explicit sense of contamination in children with ASD would be developed, as possible tools for families and experts to teach to these children what is worth eating and what needs to be avoided because it's been contaminated and therefore it's dangerous for their health.

6.7 Conclusions

This thesis investigated how access to language affects contamination sensitivity by studying food rejection behavior in children with ASDs. This method is quite new in the field, since there is only one study so far showing a lack of contamination sensitivity in this clinical population. Our studies confirmed Kalyva's study (2009), indicating that children with ASD are at greater risk of being impaired in contamination sensitivity compared to controls, despite intact cognitive abilities which have been shown to be necessary to reason about contamination in food. However, a deficit in auditory processing was consistently found in children with ASD. who lack contamination sensitivity across three of the four studies of this Thesis.

Moreover, in Study 4, these children were shown to be indifferent to linguistic information about contaminants. Taken together, these findings seem to indicate that language plays at major role in the development of contamination sensitivity in children.

To exclude any possible interference due to the use of a behavioral task in ASD due to difficulties in language and communication and executive control, we developed a non-verbal paradigm in which implicit measures of contamination sensitivity were employed. Study 3 and Study 4 showed that children with ASD who lack contamination sensitivity in a behavioral task also lack an implicit sense of contamination. These results indicate that learning processes about contamination have been compromised very early on in development, even at a fundamental and intuitive level.

The use of a nonverbal task to evaluate contamination sensitivity is also new in the field, and opens up new possibilities to test contamination sensitivity in individuals who are known to be impaired in language and communication. Differences in contamination sensitivity were not related with differences in parent's perceptions of disgust, as evaluated by the Disgust Sensitivity Scale.

Finally, for the first time in this thesis, GI symptoms in ASD have been investigated in relation to contamination sensitivity. Even though no significant differences have been found between TD children, children with ASD with and without contamination sensitivity, the latter were rated by their parents as less aware about disgust in daily life. Thus, it seems that they need more help to understand what is edible and what is not and more supervision in order to avoid disease in their natural environment. For this reason, more research is needed to better understand the biological knowledge in these children and how it could be translated into effective, developmentally appropriate disease-avoidant behaviour.

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APPENDIXES

Appendix 1 – Questionnaire about disgust sensitivity and GI symptoms in children

Appendix 2 – The DS-R (Disgust Scale-Revised)

APPENDIX 1

QUESTIONNAIRE ABOUT DISGUST SENSITIVITY IN CHILDREN

Please indicate how true are the following statements about your child.

Please write a number (0-4) to indicate your answer:

0 = Strongly disagree (very untrue about my child)

1 = Mildly disagree (somewhat untrue about my child)

2 = Neither agree nor disagree

3 = Mildly agree (somewhat true about my child)

4 = Strongly agree (very true about my child)

____1. He/she might be willing to try eating monkey meat, under some circumstances.

____2. It bothers him/her to hear someone clear a throat full of mucous.

____3. He/she never let any part of his/her body touch the toilet seat in public restrooms.

____4. He/she would go out of his/her way to avoid walking through a graveyard.

____5. Seeing a cockroach in someone else's house doesn't bother him/her.

____6. If he/she sees someone vomit, it makes him/her sick to his/her stomach.

____7. He/she probably would not would not go to his/her favourite restaurant if he/she found out that the cook had a cold.

____8. It would bother him/her to see a rat run across his/her path in a park.

____9. Even if he/she was hungry, he/she would not drink a bowl of his/her favourites soup if it had been stirred by a used but thoroughly washed flyswatter.

1. Does your child has (or had in the past) any gastrointestinal disorder? ☐ yes ☐ no

2. Had the gastrointestinal disorder been brought to the attention of a medical professional?

____

5. Had the gastrointestinal disorder been or is currently under treatment?

____

APPENDIX 2

The DS-R (Disgust Scale-Revised)

by Haidt, McCauley, & Rozin, 1994 (modified by Olatunji et al., 2007).

Please indicate how much you agree with each of the following statements, or how true it is about you. Please write a number (0-4) to indicate your answer: 0 = Strongly disagree (very untrue about me); **1** = Mildly disagree (somewhat untrue about me); **2** = Neither agree nor disagree; **3** = Mildly agree (somewhat true about me); **4** = Strongly agree (very true about me).

____1. I might be willing to try eating monkey meat, under some circumstances.

____2. It would bother me to be in a science class, and to see a human hand preserved in a jar.

____3. It bothers me to hear someone clear a throat full of mucous.

____4. I never let any part of my body touch the toilet seat in public restrooms.

____5. I would go out of my way to avoid walking through a graveyard.

____6. Seeing a cockroach in someone else's house doesn't bother me.

____7. It would bother me tremendously to touch a dead body.

____8. If I see someone vomit, it makes me sick to my stomach.

____9. I probably would not go to my favorite restaurant if I found out that the cook had a cold.

____10. It would not upset me at all to watch a person with a glass eye take the eye out of the socket.

____11. It would bother me to see a rat run across my path in a park.

____12. I would rather eat a piece of fruit than a piece of paper

____13. Even if I was hungry, I would not drink a bowl of my favorite soup if it had been stirred by a used but thoroughly washed flyswatter.

____14. It would bother me to sleep in a nice hotel room if I knew that a man had died of a heart attack in that room the night before.

How disgusting would you find each of the following experiences? Please write a number (0-4) to indicate your answer: 0 = Not disgusting at all; **1** = Slightly disgusting; **2** = Moderately disgusting; **3** = Very disgusting; **4** = Extremely disgusting.

____15. You see maggots on a piece of meat in an outdoor garbage pail.

____16. You see a person eating an apple with a knife and fork

____17. While you are walking through a tunnel under a railroad track, you smell urine.

____18. You take a sip of soda, and then realize that you drank from the glass that an acquaintance of yours had been drinking from.

____19. Your friend's pet cat dies, and you have to pick up the dead body with your bare hands.

____20. You see someone put ketchup on vanilla ice cream, and eat it.

____21. You see a man with his intestines exposed after an accident.

____22. You discover that a friend of yours changes underwear only once a week.

____23. A friend offers you a piece of chocolate shaped like dog-doo.

____24. You accidentally touch the ashes of a person who has been cremated.

____25. You are about to drink a glass of milk when you smell that it is spoiled.

____26. As part of a sex education class, you are required to inflate a new condom, using your mouth.

____27. You are walking barefoot on concrete, and you step on an earthworm.

To calculate your score: First, put an X through your responses to items 12 and 16 (these items don't count). Then "reverse" your score on items 1,6, and 10 by subtracting what you wrote from the number 4, and write those numbers in the margin. Finally, add up your responses to all 25 items (using your "reversed" scores on 1, 6, and 10). The total will be a number between 0-100.

For more information: http://people.virginia.edu/~jdh6n/disgustscale.html