RESTING STATE fMRI STUDY OF THE OLFACTORY REGION IN AUTISM

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I would like to dedicate this to my family. Thank you for your constant support.

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Thank you,

Madlyn Lawrence

ABSTRACT

MADLYN LAWRENCE: The Olfactory System in Autism: A Resting state fMRI study of the olfactory region in Autism
(Under the guidance of Dr. Tossi Ikuta)

This thesis was conducted to further the investigation of the Olfactory system of a typically developing individual compared to an individual with Autism. The Olfactory system is unique in that it is the only sensory system that is not relayed through the thalamus in the brain. Autism Spectrum Disorder (ASD), also known as Autism, is a developmental disorder which impairs a person's social, behavioral, developmental, cognitive and psychological aspects. Autism Spectrum Disorder can present with symptoms such as difficulty communicating, difficulty with social interactions obsessive thoughts and compulsions and repetitive behaviors. Subjects with Autism Spectrum Disorder present with an inability to process olfactory processes accurately compared to the typically developing (control) subjects. The results of this study can be used to further the understanding of the brain of a person with ASD in conjunction with developing treatments to increase the quality of life and neurological development.

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LIST OF ABBREVIATIONS

ABIDE	Autism Brain Imaging Data Exchange
AFNI	Analysis of Functional NeuroImages
ASC	Autism Spectrum Conditions
ASD	Autism Spectrum Disorder
APC	Anterior Piriform Cortex
BOLD	Blood Oxygen Level Dependent
FSL	FMRIB Software Library
ITI	Inter -trial interval
NAA	N-acetylaspartate
ORN	Olfactory Receptor Neurons
OXT	Oxytocin
OXRT	Oxytocin Receptor Gene
PET	Positron Emission Tomography
PN	Pyramidal Neurons
POC	Primary Olfactory Cortex
ROI	Region of Interest
TD	Typically Developing

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Literary Research Of Autism

Autism Introduction

Autism spectrum disorder is a neurodevelopmental disorder. This disorder is heterogeneous and behaviorally defined and occurs in 1 in 150 children (Amaral, 2008). "Although individuals with ASD are very different from one another, the disorder is characterised by core features in two areas—social communication and restricted, repetitive sensory-motor behaviours—irrespective of culture, race, ethnicity, or socioeconomic group" (Lord, 2018). To further understand the development of the brain in a child with ASD, a study was conducted that compared the neuroanatomy of a TD child, a child with ASD and a developmentally delayed child. Using 3-D imaging of the cerebellum, cerebrum, amygdala, and hippocampus the images were analyzed based on age and sex. "Children with ASD were found to have significantly increased cerebral volumes compared with TD and DD children. Cerebellar volume for the ASD group was increased in comparison with the TD group, but this increase was proportional to overall increases in cerebral volume" (Sparks, 2002). This study was not longitudinal nor did it conclude the neural mechanisms when dealing with ASD. Instead, these basic findings suggest abnormal brain developmental processes early in the clinical course of autism (Spark, 2002).

The exact genetic cause of the disorder has yet to be determined. Some believe there to be an epigenetic overlap that may increase or decrease the severity of the developmental disorder. ASD has been a growing topic studied by researchers. There have been several studies conducted to narrow down the genetic and epigenetic complements. Deletion, Duplication, translocation and other copy number variations (CNV) of chromosomes in individuals with ASD have been identified. These findings have not been used to conclude an exact etiologic role (Marshall, 2008). "On the basis of numerous studies that have been undertaken to elucidate the pathogenic

mechanisms underlying ASD, it is widely accepted that ASD is a disorder with strong genetic components. However, autism is an etiologically heterogeneous disorder in that no single genetic mutation accounts for more than 1–2% of ASD cases" (Abrahams and Geschwind, 2008). As previously mentioned, ASD has a few major defining characteristics. The defining behavioral characteristics of individuals with autism spectrum disorders (ASD) can be categorized into three main groups. The areas of deficit include social relatedness, communication skills, and display of stereotyped behavior such as narrow interests and activities (American Psychiatric Association 2000).

Mirror Neuron Theory

Hans Asperger was one of the first to note the 'abnormal' reactions to sensory stimuli that individuals with ASD often display. He emphasized those concerning touch, smell, and taste (Wicker, 2016). One possibility for the neural disturbance may be a recently discovered class of neurons, mirror neurons (MN) Located in the frontal cortex, they show a relationship to actions performed by the self and actions performed by others. This provides a potential bridge of the gap between two minds (Williams, 2001). "Recent evidence of impairments in action understanding in persons with autism may be associated with atypical functioning of the MNS in this population. Understanding an action may involve two important aspects: (a) comprehending the motor action (what), and (b) inferring the intention behind the action (why)" (Kana, 2011). A third recently identified pathway projects from the visual centers to the inferior parietal lobe. This region has been referred to as the "emotional pathway" because it projects into the limbic pathway. Rich in mirror neurons, any damage or dysfunction of this pathway could lead to a decrease in social empathy (Brang, 2008).

An MRI report that studied an individual with autism suggests an additional congenital aplasia.dysplasia of the olfactory bulbs. The reduction of vasopressin and oxytocin receptor binding being the consequent (Brang, 2010). "The oxytocin receptor gene (OXTR) is a high-affinity G-protein-coupled receptor encoded by the OXTR gene located on human ch 3p26.2. It binds oxytocin (OXT), a nine-amino-acid neurohypophyseal hormone encoded by the OXT gene" (Geracioti Jr, 2009). This neuromodulator is high in abundance in the amygdala. Since it is heavily involved in physiological processes (e.g., breastfeeding, birthing process) it is known to play a role in social cognition (Carter, 2007; Carter et al., 2008). In a study conducted on mice, OXT or OXTR knockout mice display impaired social memory, while parturition is largely unaffected (Ferguson et al., 2000; Takayanagi et al., 2005). In this experiment male mice with the OXTR knockout are unable to recognize their own species. This suggests that females may have a mechanism that compensates for the knockout gene (Sun et al., 2008). "Abnormal neuropeptide processing in autistic children, yielding reduced OXT blood levels despite enhanced concentrations of OXT precursor (Green et al., 2001; Modahl et al., 1998), may further exacerbate this deficit, bringing OXT signaling below a critical threshold necessary for the physiological development of social behavior" (Geractioti Jr, 2009).

An Individual with ASD and the Olfactory System

The effects of olfaction processing in an individual developing with Autism Spectrum Disorder (ASD) has been studied while being compared to typically developing individuals (TD). There has been evidence suggesting atypical sensory and, specifically, olfactory processing is present in neurodevelopmental conditions, including autism spectrum disorders (ASDs) (TONACCI, 2018). "Autism spectrum (AS) conditions, like autism and Asperger syndrome, are characterized by atypical socio-communicative functions, restricted interests, and repetitive

behaviours. In autism, but not in Asperger syndrome, early cognitive and/or speech delays or atypicalities are also part of the diagnostic criteria" (APA 2000). Individuals with autism spectrum disorder can also be characterized as displaying behaviors that are not seen in those classified as typically developing. There is often intense fixation on perceptual features as well as atypical perceptual processing (Galle, 2013).

In order to adequately understand neurological disorders such as autism; it is vital to determine the significance of the olfactory pathway in the disorder. One approach to further the understanding of sensory dysfunction in autism would be to examine the integrity of specific sensory systems. Studies conducted previously have had inconclusive results. Focusing on a response pattern across distinct modalities can help to clarify discrepancies. Another direction to establish links between behavioral responses and neurobiology in autism is to study the chemosensory processing in the brain (Bennetto, 2007).

Since the olfactory pathway has been extensively studied more is known about the pathway in a typically developing brain. This can be used for comparison. "Signals From olfactory receptors converge via the olfactory nerve in the glomeruli of the olfactory bulbs where numerous inhibitory acting interneurons [mainly gamma aminobutyric acid (GABA) and dopamine] are engaged in filtering and amplifying odour signals" (Schecklmann, 2013). These neurotransmitters allow for levels of discrimination of olfactory sensitivity to be examined. Due to the piriform cortex being connected to the anterior olfactory nucleus, parts of the amygdala and the entorhinal cortex being directly connected to bulbs; this means these parts of the brain are connected to several regions of the brain. These other regions include orbitofrontal cortex, hippocampus, insula, cingulate cortex, basal ganglia, hypothalamus, and the thalamus (Schecklmann, 2013).

With this pathway in mind, several neurobiological studies have been conducted on children with autism to determine if it is possible that there is a taste dysfunction or the inability to identify specific tastes and smells correctly. The evidence of brainstem dysfunction in autism is apparent. The dysfunction presents as hypoplasia of facial nerve (CN VII). This particular nerve carries information from two-thirds of the anterior portion of the tongue. Any damage to this pathway affects taste detection (Bennetto, 2007).

Olfactory Introduction

Over the past decade, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have begun to detail the functional neuroanatomy of the olfactory system (Zaid, 2000). Changes in blood oxygen level dependent (BOLD) are measured by fMRIs. The fMRI sends a signal to assess the 'functional status' of different brain regions (Zaid, 2000). The BOLD signal is a representation of the change in the proportion of oxyhemoglobin and deoxyhemoglobin. The fluctuation that occurs is due to a brain region increasing its blood flow during increased neural activity (Ogawa et al., 1990, Ogawa et al., 1992). The piriform region is at the junction between the frontal and temporal lobes. The primary olfactory cortex (POC) is located within the piriform region (Price, 1991). More specifically, the piriform cortex wraps around the junction and extends into the anterior part of the temporal lobe's medial wall. This is the region where researchers were first able to visualize odorant-induced activity in the piriform cortex of humans (Zaid, 2000). "A central feature of odor perception is its hedonic or affective component. Most odors are labeled as "pleasant" (positive hedonic value) or "unpleasant" (negative hedonic value), and recent functional neuroimaging studies performed on humans have successfully demonstrated that the valence of odors is represented in particular in the orbitofrontal cortex" (de Araujo, 2005).

Materials and Methods

Data Acquisition

Autism Brain Imaging Data Exchange (ABIDE) was used to collect MRI images, clinical and demographic data. New York University served, among image data obtained from several sites, served as the cohort for this study. 120 individuals for whom resting state as well as structural data was available. Of these individuals, 60 were diagnosed with ASD (hereafter ASD group, 14.57±7.00 years old) and 60 were TD age-matched individuals (control group, 15.81±6.25 years old). Analyses of this study were approved by the Institutional Review Board of the University of Mississippi.

Resting state echo planar image (EPI) volumes had 33 slices of 4mm 64x80 matrix with 4mm thickness (voxel size = 3x3x4mm), with repetition time (TR) of 2000ms and echo time (TE) of 15ms. A total of 180 volumes (5 minutes) were used in the analysis. High-resolution structural T1 (MPRGE) volumes were acquired as 128 sagittal slices of 256mm x 256mm with 1mm thickness (voxel size = 1.3x1x1.3mm, TR=2530ms and TE=3.25ms).

Data Processing

FMRIB Software Library (FSL,) as well as Analysis of Functional NeuroImages (AFNI) was used to conduct the data processing and statistical analyses in this study. Each subject's skull was stripped, segmented (gray matter, white matter and CSF) and registered to the MNI 2mm standard brain to accurately obtain the anatomical volume. To begin with, four EPI volumes were removed. De-spiking the interpolation removed transient signal spikes. Correction of head motion was accomplished by the volumes being linearly registered to the first volume. From here, six motion parameters and the displacement distance between two consecutive volumes were estimated. Resting state volumes were regressed according to white matter, cerebrospinal fluid signal fluctuation and six motion parameters. Smoothing with a 6mm FWHM Gaussian kernel allowed for the volumes to be resampled. Then the volumes were spatially transformed and aligned to the MNI 2mm standard brain space. In order for the processed EPI volumes to be registered to the MNI space, 12 affine parameters were created between rs-fMRI volumes and MNI152 2mm space. The root mean square deviation was calculated from motion correction parameters, at an *r=40mm* spherical surface using FSL's *rmsdiff* tool (Power et al. 2012, 2015). To calculate these values scrubbing was performed where volumes with excess motion were removed as a displacement between two EPI volumes. Displacement distance volumes that exceeded 0.3mm were removed (i.e., scrubbed) from further statistical analyses (Siegel et al. 2014). The olfactory bulb, olfactory tract, anterior piriform cortex, and posterior piriform cortex segmentation in the MNI 2mm standard space (Figure 1) is described in the previous study (Kiparizoska and Ikuta 2017). In each region of interest, an analysis of voxel-wise connectivity was conducted. Time course for each ROI is spatially averaged. Correlations can then be tested because each ROI is registered to the respective EPI space and each individual voxel across the

brian. Correlations between the ROI and voxel was used for group level analysis and represented by a Z-score following registration of the MNI 2mm brain space.

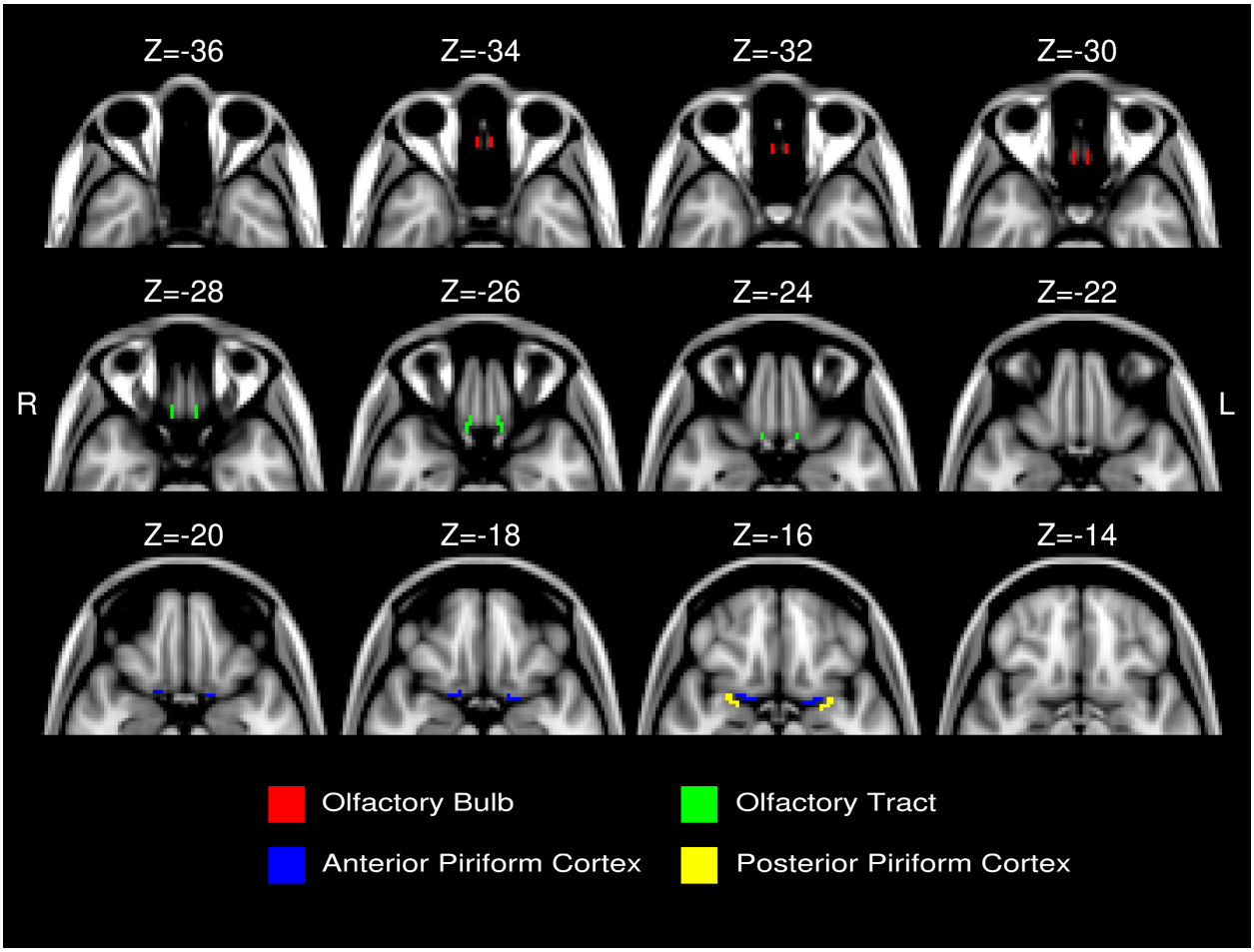

Figure X: Four olfactory seed regions (adapted from Kiparizoska and Ikuta 2017).

Results

The voxel-wise analysis of functional connectivity to/from the anterior piriform cortex showed significantly lesser connectivity to the ventromedial prefrontal cortex in the ASD group compared to the control group (peak MNI=[-4 +36 -24] 98 voxels; Fig 2). No region indicated greater connectivity with the APC in the ASD group than in the control group. None of the three other seed regions showed significantly different connectivity between the two groups.

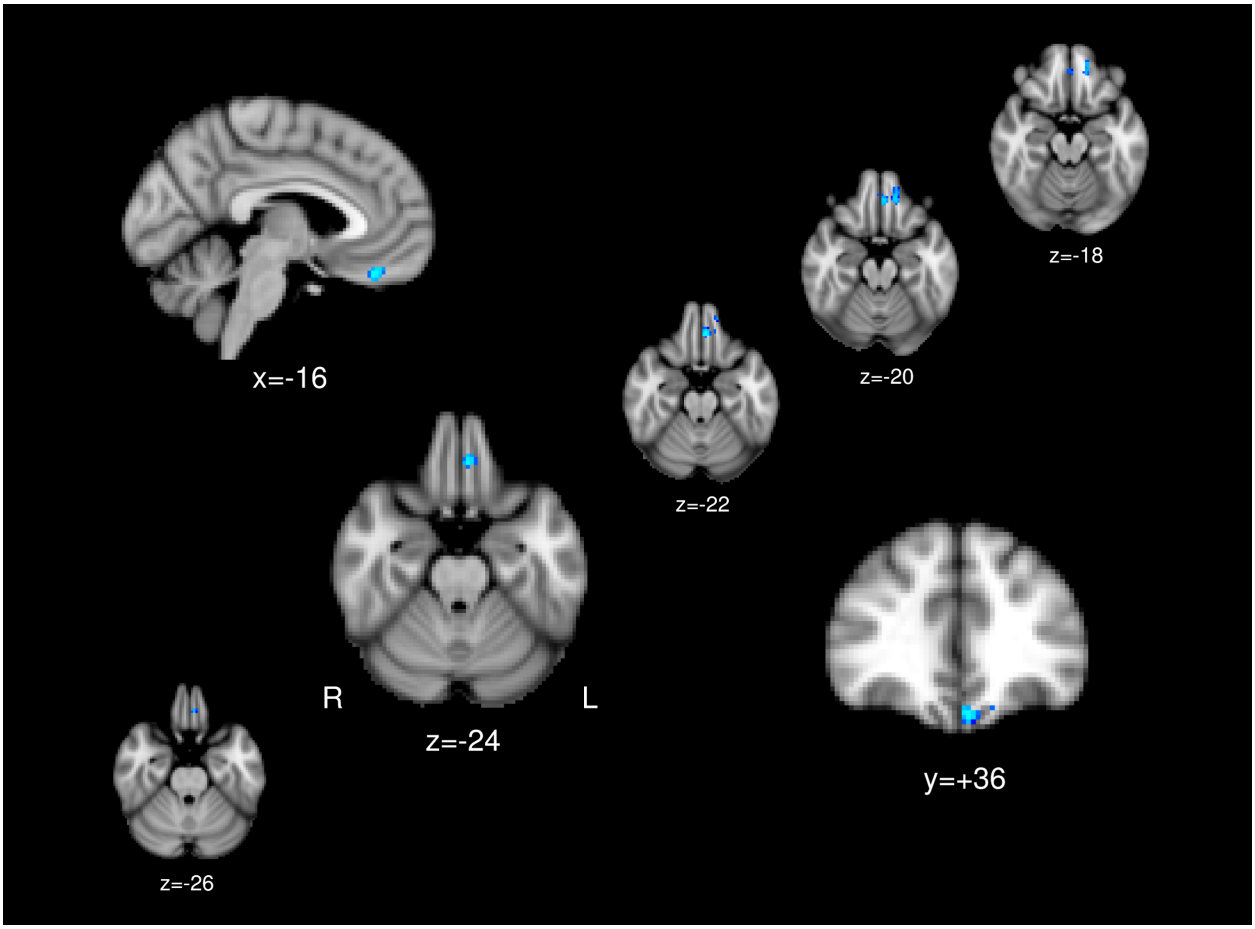

Figure 1: Contrast of APC connectivity between the ASD and control groups. Blue region indicate less connectivity to the APC in ASD group.

Discussion

In this study, we aimed to identify differential functional connectivity of the olfactory regions in ASD compared to typically developing cohort. The results of our study indicated the ventromedial prefrontal cortex (vmPFC) showed less connectivity to the anterior piriform cortex (APC), compared to the control group. The results may suggest that there is a correlation with self-related processing and values in decision making connectivity to the APC.

In order to assess how the vmPFC connectivity affects the olfactory system, a person with ASD's brain, the corresponding parts of the brain must be examined. Through our resting state fMRI, we found that there was less connectivity between the vmPFC and the APC. The anterior piriform cortex is found in the cerebrum and one of the primary recipients of olfactory projections in the brain. Based on our findings, the functions of each of these regions of the brain must be examined in order to determine how the connectivity would affect the brain of an individual with autism spectrum disorder.

The Anterior Piriform Cortex

The processing of olfactory information is an integral part of how most vertebrates rely on the processing of sensory information. The olfactory system has the same scheme across species. Only two synaptic relays to reach the olfactory cortex from the outside world. The piriform cortex, (one of the first cortical destinations of olfactory information in mammals) is a primitive paleocortex. Being that it is a crucial brain region for the synthetic perception of odors (Bekkers and Suzuki, 2013). Olfactory receptor neurons (ORNs) are the first part of the 'wiring diagram' in the olfactory circuit. They are located in the main olfactory epithelium (Osmanski, 2014).

A study was conducted to investigate the odor-evoked pathway using functional ultrasound imaging in rodents. This study was used to analyze how odor was processed in the

main olfactory bulb (MOB) and anterior piriform cortex. Changes in blood volume detected by an ultrasound when the brain is activated has been observed in certain rodents that express a certain olfactory receptor (Firestein, 2001, Mombaerts, 2004, Mori and Sakano,2001), suggesting that this certain receptor projects to the four glomeruli in the MOB. This first central rely is responsible for the coding of olfactory information (Shepheard and Greer, 1998). Osmanski used this information to later conclude the MOB sends information to the APC, which is the main output structure of the primary olfactory cortices (Osmanski, 2014). Functional ultrasound (fUS) imaging ((Macé et al., 2011) has been a recently developed technique used to detect activity in the APC due to odor-evoked stimulation (Osmanski, 2014). A modified version of a multi vial perfusion system (ValveBank 8 II, AutoMate Scientific; USA) was used as an olfactometer being that it was attached to an air compressor (Osmanski, 2014). "All rats were anesthetized by an intraperitoneal (i.p.) injection of a mixture of ketamine (60 mg/kg, Imalgene 500®, Merial; France) and medetomidine (0.4 mg/kg, Domitor®, Pfizer Santé Animale; France)" (Osmanski, 2014). The diluted odor (50 μL) was placed on filter paper and loaded into a syringe reservoir. The pressure controlled system ensured each rat had a constant rate of odor streaming into the animal's nostrils (Osmanski, 2014). "A single activation trial lasted for 48 s. After 6 s of baseline recording under a constant deodorized airflow, one of the two odorants, hexanal 1% or pentylacetate 1% (Sigma-Aldrich; USA), was delivered for 15 s. Another 27 s of air delivery was allowed to recover the baseline value of the metabolic signal" (Osmanski, 2014). The olfactory system is hypersensitive to desensitization habituation. To circumvent this problem the experiment allowed 3 minutes of inter- trial interval (ITI). In the main olfactory bulb both odorants used created specific and symmetrical maps. The distinction made between the two scents was that Hexanal created dorsal MOB activity when compared to pentyl acetate in the

same rat. When observing the APC region the pattern of activation was consistently global and widespread throughout all of the layers covered by odor activation (Osmanski, 2014)

The fUS was used to probe activation at the macroscopic level. It was demonstrated that the spatial organization throughout the MOB is not conserved in the APC. There is widespread activation in the APC in response to an odor. This was not distinguishable between different odors (Osmanski, 2014). The fUS was used to follow olfactory activation. This study showed the aPC demonstrated widespread response to different odors (Osmanski, 2014).

To further understand the anterior piriform cortex and its function another study was conducted that studied mice in the awake state as opposed to under anesthesia. By using single-cell recordings from head-restrained awake mice, researchers were able to study the odor response profiles of single neurons throughout the anterior piriform cortex (Zhan, 2010). For this experiment, the neurons were labeled and the cells were distinguished based on morphology and neurotransmitter phenotypes. A recording from an awake animal compared to an anesthetized one has many benefits. The side effects of anesthetics are removed. Also, the juxtacellular labeling allows for cells classification of the cells based on the morphology (Zhan, 2010).

A head plate with a 4-mm-diameter hole on the skull was centered over the APC. The plate was fixed to the skull of the rat with stainless steel screws and dental cement. A portion of the skull (4 mm hole) was exposed, thinned but not broken. A recovery period of 3-5 days was allowed for the mice before recording began. Once the recordings began, a small hole (200–300 μm) was made in the skull. This allowed for electrodes to be lowered toward the APC for recording (Zhan, 2010). "Odor stimuli consisted of 24 monomolecular chemical compounds: ethanol, cyclohexanol, 2-decanol, 3-methylbutanol, ethyl acetate, ethyl butyrate, 2-phenethyl acetate, *N*-amyl-acetate, 2-methyl-butyraldehyde, heptanal, citral, benzaldehyde, isophorone,

geranyl acetone, 1-pentanol, 2-pentanone, benzene, toluene, styrene, benzyl ether, 2,5-dimethylpyrazine, 2′-hydroxyacetophenone, propiophenone, and acetophenone" (Zhan, 2010). In all four trails each odorant was delivered and each of the 24 odors were delivered consistently with air control to all cells. The residual odorants were minimized from previous trials by an additional stream of purified air (100 ml/min). This air was injected into the final valve after each odorant was presented for 3 seconds (Zhan, 2010).

Among pyramidal neurons (PNs) a noticeable difference in variability of selective patterns was found. When dissimilar odorants were used about one-fourth of the PNS. showed broad activation. A subpopulation of PNs did exhibit broad inhibition. When examining GABAergic neurons, it should be noted that they exhibited nonselective excitatory responses to test odorants and rarely exhibited inhibition. Non-GABAergic nonpyramidal neurons within the deep layers generally showed to exhibit a stronger inhibition by several different odorants (Zhan, 2010). Recorded in this study were specifically labeled cells. These included: 57 APCX cells, including 40 PNs, 11 non-GABAergic nPNs, and 6 GABAergic cells The individual cells were each tested with 24 odorants. Which in turn produced a total of 1368 odor–cell pairs. (Zhan, 2010). The results of this study were consistent with those ran with anesthesia and animals with electrophysiological recordings/optical imaging from calcium signals (McCollum et al., 1991; Schoenbaum and Eichenbaum, 1995; Rennaker et al., 2007; Poo and Isaacson, 2009; Stettler and Axel, 2009; Isaacson, 2010). This suggests that the results of the study conducted with anaesthetised rats were consistent. The results of the Zhan study suggested odor activation in awake rats was accomplished by broad and narrow pyramidal cells in the APC. It was suggested that several types of interneurons played roles in the processing of olfactory information (Zhan, 2010).

The activation of the anterior piriform cortex in the previous two studies has proven that at the macroscopic and microscopic levels of this region of the cortex is complex. The type of processing that occurs in the anterior region of the piriform cortex is an integral part of not only processing in the olfactory pathway but in the process of learning.

The neurological mechanism involved in facilitating food selection is not fully understood. Flavor aversion learning (FAL) and conditioned flavor preference (CFP) play a role in how an animal selects food. Using a Fos immunohistochemistry, the neuroanatomical structures of the CFP preferences were analyzed (Mediavilla, 2016). "Rats were trained over eight alternating one-bottle sessions to acquire a CFP induced by pairing a flavour with saccharin (grape was CS+ in Group 1; cherry in Group 2; in Group 3, grape/cherry in half of animals; Group 4, grape/cherry in water)" (Mediavilla, 2016). Saccharin, which has no nutritious properties, was used so that flavor-taste preference would be solely based on hedonic taste olfactory mechanisms. A grape flavor was offered to each animal following the training day. Their brains were then analyzed and processed for c-Fos. Neurons that showed reactivity for Fos-like immunoreactivity were counted and made note of in three areas: the infralimbic cortex, nucleus accumbens core, and anterior piriform cortex (aPC) (Mediavilla, 2016).

The amount of activated cells in the anterior piriform cortex is increased when flavor-taste preference is induced . The is considered the first relationship between the APC and CFP. (Mediavilla, 2016). The immunohistochemical analysis demonstrated a significant difference in the number of cells in the APC that were c-Fos-labelled during the procedures. The initially neutral flavor eventually gained a motivational significance. This caused a manifestation of flavor preference in groups 1 and 2. This preference was demonstrated in an increase in activity in the APC. Group 4 had no increase in APC activity. This was due to the two flavors being diluted in

water. Since the flavors were equally preferred the rats were unable to create flavor- taste learning. The outcome of group 4 allows for the possibility of the increased cellular activity in the APC to be attributed to sensorial processing to be ruled out Mediavilla, 2016).

One of the many connections the anterior piriform cortex has that is imperative in this study is to the ventromedial prefrontal cortex. As previously mentioned, the vmPFC is known to be involved in social and emotional processing. The APC has been studied and shown to harbor cells that demonstrate plasticity. Learning cannot occur without the ability to retain and build upon information presented to these cells in the olfactory pathway. Less connectivity between the APC and the vmPFC in a person with ASD means less of an ability for selectivity in processing of not only olfaction but perception of emotions.

The vmPFC

Decision making often occurs in the face of uncertainty about whether one's choices will lead to benefit or harm. The somatic-marker hypothesis is a neurobiological theory of how decisions are made in the face of uncertain outcome. This theory holds that such decisions are aided by emotions, in the form of bodily states, that are elicited during the deliberation of future consequences and that mark different options for behavior as being advantageous or disadvantageous (Naqvi, 2006). One of the most important regions of the brain that is crucial for goal-directed behavior is the orbitofrontal cortex (OFC) (Howard, 2017). Goal directed behavior towards rewards (e.g. food, shelter, and water) is a central function of the brain. The mechanisms of behavioral control that allow for adaptations to changes in how the subject values these rewards was studied by Balleine and Dickinson (Balleine and Dickinson, 1998; O'Doherty et al., 2017). When compared to the OFC, activity in the vmPFC has been shown to reflect decision values. This occurs regardless of reward identity (Plassmann et al., 2007; Chib et al., 2009;

Lebreton et al., 2009; Levy and Glimcher, 2011; McNamee et al., 2013; Howard et al., 2015) suggesting that the identity-specific signals processed in the OFC are directly updated there. The input they provide is crucial to the decision values made in the vmPFC (Howard, 2017).

A study was conducted to investigate the identity-specific signals that are directly updated in OFC to prove that they provide critical input to representations of decision values in vmPFC. "Nineteen healthy human participants with no history of psychiatric illness (seven male; age, 20–34; mean ± SD, 25.0 ± 3.45 years) gave informed written consent to participate in this study. Eight food odors, including four sweet (strawberry, caramel, cupcake, gingerbread) and four savory (potato chips, pot roast, sautéed onions, garlic), were provided by International Flavors and Fragrances" (Howard, 2017). This experiment was conducted with a custom-built olfactometer that was controlled by a computer. The odors were delivered to the participants noses with precise and consistent flow rate (3.6L/min). Each odor was administered through amber bottles with liquid food odors. Three days of testing were conducted. Each day the participant was instructed to arrive hungry. They must fast for six hours before testing. Visual analog scales were used with a scroll wheel and mouse button press to rate all behaviors by participants. (Howard, 2017).

When looking at how the stimulus and expected value options are represented in the brain the key region to focus on is the vmPFC (Fellows, 2007). "Recent fMRI studies building on connections between VMPFC and decision making (Sommer et al., 2009, Liu et al., 2007, Chua et al., 2009, Ursu and Carter, 2005) have reported distinct activation patterns in the medial and lateral OFC during periods of regret" (Levens, 2014).

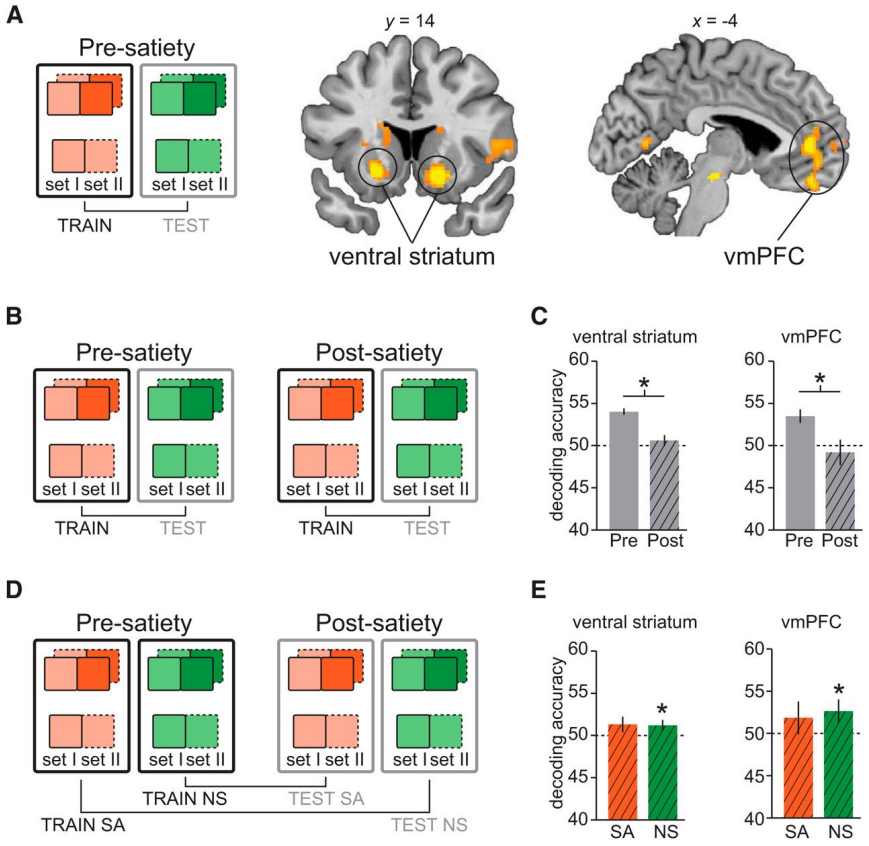

Figure 2.

Neural representations of general reward value. *A*, The classifier was trained to discriminate [high versus low] versus [low versus low] offer- or choice-related fMRI activity for one odor identity and tested on the same conditions but evoked by the other identity. Classification was performed first on presatiety data to identify regions that encoded the general value of the expected reward outcomes. Effects that survived small-volume correction for multiple comparisons using a priori regions are shown here. *B*, *C*, General value decoding was performed separately in the presatiety and postsatiety data (*B*), and resulting accuracies were averaged across voxels in VS and vmPFC clusters (*C*). *D*, A classifier was trained to discriminate [high versus low] versus [low versus low] offers or choices for one identity in presatiety and tested on the same conditions but for the other identity in the postsatiety data. *E*, Accuracies were averaged across voxels in the VS and vmPFC clusters. Error bars depict mean and SEM for $n = 17$. *$p < 0.05$, *t* tests.

The vmPFC in Autism

Self-related processing involves the ventromedial prefrontal cortex (vmPFC). A disruption in this region is often associated with disruptions in emotional and social functioning. These types of disruptions have been observed in depression and autism (Kim, 2014). To Further investigate the function of the vmPFC studies have been conducted.

As previously mentioned the hormone oxytocin may have a significant impact on the olfactory pathway. Several studies have been conducted to better the understanding of the connectivity and function of the ventromedial prefrontal cortex in a child with ASD compared the typically developing cohort. The key question is exactly how patterns of neural activation in the moral network might differ when processing these varied classes of moral challenge (Kim, 2014). "One possibility is that network activation will only differ as a function of the different cognitive parameters recruited (i.e. conflict resolution, engagement of systems involved in deliberative reasoning). If this were the case, difficult moral decisions may only differ from easy moral decisions in their recruitment of the dlPFC and ACC (Greene *et al.*, 2004)" (FeldmanHall, 2013).

One neuropeptide in particular, oxytocin, could be an effective therapeutic strategy for the social and communication deficits associated with autism. As these deficits are currently untreatable this peptide has been researched further (Aoki, 2015). Oxytocin has been found to promote aggression and antisocial behaviors under certain circumstances (Guastella, 2012; Striepens,2012; Miller 2013) other studies have found evidence to conclude the neuropeptide can have an enhancing effect on social behaviors in disorders like ASD (Gordon 2013;Bakermans-Kranenburg, 2013; Meyer- Lindenberg; 2011, Van, 2012; Yamasue, 2012; Veening, 2013; Tachibana, 2013).

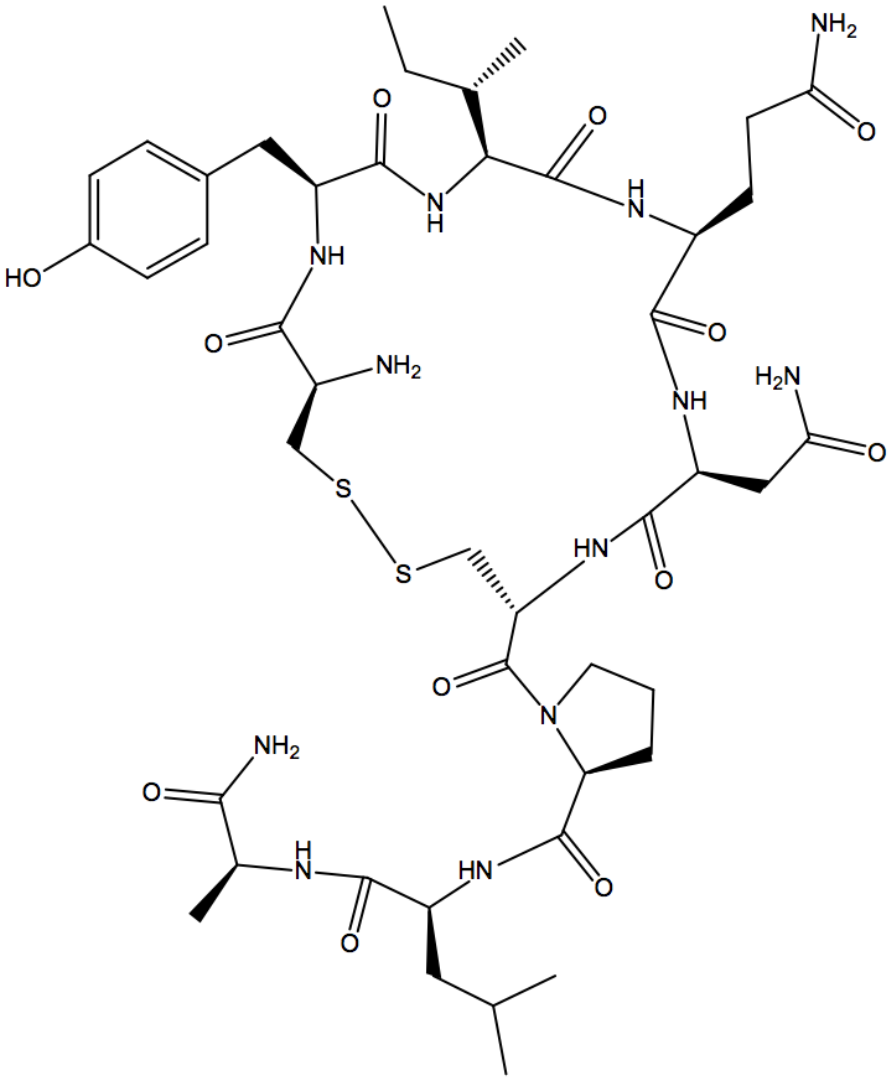

Figure Y: Chemical Composition of the hormone Oxytocin

Oxytocin and its potential therapeutic effects on the social deficits in ASD were evaluated in a double-blind experiment. This randomized controlled trial was run with 40 high-functioning men diagnosed with autism. A single dose of Oxytocin would be administered and evaluated with functional magnetic resonance imaging (fMRI) and 1H-magnetic resonance spectroscopy (1H-MRS) (Aoki, 2015). Previous studies have been found to show the vmPFC/ACC is influenced by oxytocin (Meyer-Lindenberg, 2011; Domes, 2013; Bethlehem, 2013; Tost, 2010), the current study examined vmPFC/ACC40 which involved studying the pathophysiology of distorted social cognition in individuals with ASD (Aoki, 2015).

"A path analysis was conducted to elucidate the multiple relationships between the influences of oxytocin on the N-acetylaspartate (NAA) levels, the oxytocin induced changes in fMRI signals and the effects of oxytocin on social communication behavior, with a standard maximum likelihood estimation" (Aoki, 2015). The paired t-test was unable to detect any significant difference in the quality or spatial composition between oxytocin and the placebo (P=40.088). NAA levels between oxytocin and placebo trials did not show a significant difference either ($t30 = 1.315$, $P = 0.198$). Although a significant relationship was established between the influence of oxytocin and NAA levels on oxytocin-induced changes in the vmPFC region when looking at the fMRI. These results were found through a linear regression test (Aoki, 2015).

^{1}H-Magnetic Resonance Spectroscopy with Oxytocin induced differences in fMRI

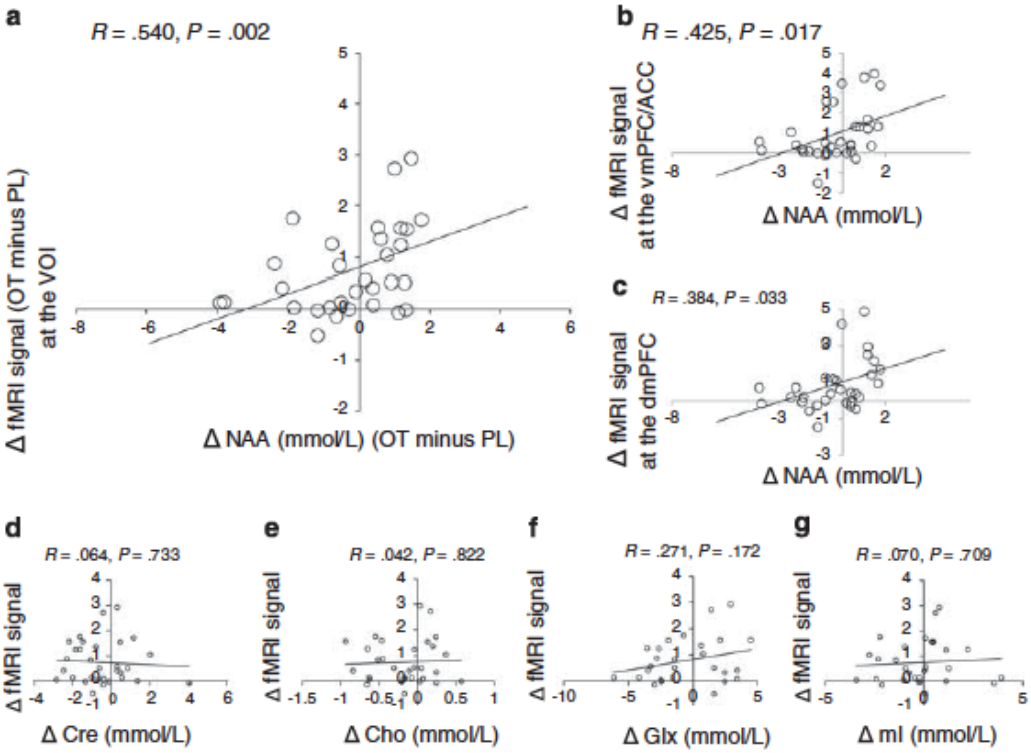

Figure 3. Relationships between the oxytocin-induced differences in ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) measures and the changes in functional magnetic resonance imaging (fMRI) signal. Scatterplots show the relationships between the oxytocin-induced N-acetylaspartate (NAA) differences (NAA levels at oxytocin (OT) sessions minus NAA levels at placebo (PL) sessions) and the task-evoked fMRI signal changes in (**a**) the volume-of-interest (VOI), (**b**) the ventromedial prefrontal cortex (vmPFC)/anterior cingulate cortex (ACC) and (**c**) the dorsomedial prefrontal cortex (dmPFC). No significant relationship was detected between the changes in the fMRI signal and the differences in (**d**) creatine (Cre), (**e**) choline-containing compounds (Cho), (**f**) glutamine and glutamate (Glx) or (**g**) myo-Inositol (mI) levels.

"The crucial finding in the present study is that the influence of oxytocin on NAA levels in the vmPFC/ACC, which is not confined to a specific psychological task, underlies the oxytocin induced increase of originally diminished task-specific fMRI signal in the same brain region"(Aoki, 2015). This finding would suggest that in the vmPFC/ACC neural mechanism of oxytocin-induced behavioral improvements could have an impact on male socio-communication deficits that are observed in people with ASD (Aoki, 2015).

Dysfunction of vmPFC may account for impaired self-referential processing in ASD. vmPFC activation of the vmPFC has been found to be absent during pragmatic language comprehension in which the control group showed activation of vmPFC (Tesnik, 2009). vmPFC has been found to show self-referential processing with a decrease in activation (Kelley et al., 2002; Mitchell et al., 2005), and when evaluated based on several types of stimuli the subjective value ranged from food to social reward (Peters and Büchel, 2010; Rangel and Hare, 2010). The weaker connectivity in the APC and vmPFC in ASD may implicate that olfactory signal may have lesser significance to self referential processing.

The common signs of ASD (which include an inability to comprehend and interact in social settings) may be compared to the connectivity of the anterior piriform cortex. The plasticity that is associated with the APC region of the brain helps encode odors and learning. The vmPFC helps with regulation of self-related processing and decision making. In the brain of a person affected by ASD, they showed less connectivity between these two integral parts of the brain. To further comprehend the mechanistic link between these two regions we turned to studies that investigated the sniff response in TD individuals and individuals with ASD.

A type of mechanism in the brain that also serves as sensory motor coordination are known as internal action models (IAMs). These brain templates allow for the initiation of

refinement of expected sensory outcomes as well as continuous refinement from sensory input (Rozenkrantz, 2015). When analyzing the human olfactory sniff response, generally, unpleasant or intense odors are associated with low-magnitude sniffs. Mild and pleasant odors are often followed by high-magnitude sniffs (Bensafi, 2003; Johnson, 2003; Frank, 2003). The sniff response is considered an IAM because it entails the two key qualifications of such a response. It contains a fine adjustment of a motor process - the sniff- which is in accordance with the sensory input - the odor. Both the fine motor and sensory input are needed to qualify as an IAM (Rozenkrantz,2015). The researchers in this experiment hypothesized, the sniff response would be altered in children with ASD. To clarify, the researchers in this study did not hypothesize children with ASD would be unable to sniff, but rather that they would generate an inappropriate sniff given a particular odor (Rozenkrantz, 2015).

The sniff-response in children was measured using a computer-controlled air-dilution olfactometer. This device was equipped with a custom-designed double-barreled pediatric nasal cannula that allowed for simultaneously delivery of odors. Nasal airflow was measured to accurately account for the sniff response following pleasant (rose or shampoo) and unpleasant (sour milk or rotten fish) odors. This specific experiment was conducted using 18 children with ASD (17 boys, mean age = 7 ± 2.3) and 18 age- and gender-matched typically developing (TD) children (17 boys, mean age = 6.7 ± 2.1) as controls. The procedure lasted 10 minutes and consisted of 20 trials (10 of each valence), each 1–2 s in duration, separated by a 30-s intertrial intervals. Four sniff parameters: sniff volume, peak airflow rate, mean airflow rate, and duration were used to be able to characterize TD and ASD sniff response (Rozenkrantz, 2015). "A multivariate repeated-measures ANOVA applied to all parameters revealed a significant interaction between odorant valence (pleasant versus unpleasant) and group (TD versus ASD)

($F_{1,34} = 4.47$, $p < 0.05$), reflecting larger sniffs for pleasant versus unpleasant odors in TD alone. In contrast, ASD sniffs did not significantly differ by odor at any point along the sniff trace" (Rozenkrantz, 2015).

To ensure that the sniff response obtained from this study was correlated to ASD classification the scores were compared to independently obtained autism severity scores (Autism Diagnosis Observation Schedule [ADOS]). The finding was that as the severity of ASD increased based on ADOS scores so did the duration of the sniff response to unpleasant verse pleasant odors. The sniff response was compared to a TD and children with ASD with multivariate normal density classifier applied to the sniff parameters. This allowed for the sniff response to be tested at a single-subject level. This type of test allowed for the results of the altered sniff response to be characterized as being evident in children with ASD (Rozenkrantz, 2015). This study can help to explain our findings of the resting state fMRI olfactory region in Autism. When analyzing this sniff response an individual with ASD demonstrated the inability to accurately control the duration of the sniff response when presented with an unpleasant odor. Sniffing is a self-related process that is controlled by decision making.

In our study, the APC showed lower connectivity to the vmPFC in the ASD group compared to the typically developing group. The vmPFC has been shown to be responsible for self-related processing and values in decision making. Disruption of the connectivity between the odor information and decision making is consistent with the molecular findings in Autism.

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