

PART 1: THE CONTEXT BEHIND MY RESEARCH

What Sparked My Interest in Autism Research

My dad's shout to come over right away was heard clear across the house. Something was going on. As I ran over, it was obvious something (or someone) was amiss. David was not in the bathroom. You see, David has been trained to go upstairs and sit on the toilet each night, following dinner. Instead, my dad saw David shuffle across the hall with his pants around his ankles. I found David, in all his glory, on his bed, leaning on both elbows and knees, staring at the calendar on the wall next to his pillow. The calendar holds mystical powers for David. It lists all the activities he's scheduled to do for the next day. David is my 15 year old brother. David has autism. While David stared at the calendar, I stared at him. That moment taught me what unconditional love means. Treatments and a cure for David lie in science. I firmly intend to be involved in this search, to improve the lives of developmentally disabled individuals.

Curiosity motivated me to initiate an autism study at a Stony Brook University research lab, "Gender Differences in Hypersensitivity Behavior in a Rat Model of Autism." In 2006, I methodologically studied male and female hypersensitivities in hyperserotonemic autistic-like and typical young adult rats. My lab work was supervised by Stony Brook University Professor, Whitaker (Ph.D. Psychology) and her research lab associates.

Growing up with an autistic brother, I was baffled by the mysterious manifestations of this disorder. Autism is a severe, life long, pervasive developmental disorder affecting social, verbal, sensory and stereotypical behaviors. I constantly ponder, "*What if my brother, age sixteen, and his autistic peers could become typical teenagers?*" Even as a child I was always eager to help my brother's tutors, hoping they'd "cure" my brother. Scientific researchers advancing humanity's wisdom pool could make my dream reality.

Hypersensitivity is an overreaction of one or more of the five senses (hearing, sight, smell, taste, touch). Hypersensitivity interferes with ability to learn, reach one's full potential, and enjoy a typical quality of life. Observing hypersensitivity in my brother and his peers, I searched for scientific articles about autistic individual's hypersensitivities affecting all five senses. I recalled observing my brother's tactile hypersensitivities. I remember watching him cringe repulsively and fearfully upon touching green vegetables and "Play-Doh," when he was a preschooler. Parents told me their auditorily sensitive autistic children couldn't tolerate firework's booming sounds. Do autistic females experience greater hypersensitivity than autistic males? Are all five senses more impaired in the autistic vs. typical individual? If there are significant sex differences, will autistic females and males share a common etiology or just similar manifestations? Investigating these curiosities was my research's mission.

My review of published research found autistic females are overall more impaired and more cognitively blighted than autistic males. Initially, my experiment studied tactile and auditory hypersensitivity in a rat autism model. Upon completing that project I sought to delve deeper into the connection between hypersensitivity and autism. In my contact with autistic individuals I noticed atypical olfactory behavior. Searching scientific literature, I found virtually no investigations of autistic individuals' olfactory hypersensitivities. The dearth of work in this area prompted me to initiate a study. I researched odors rats find pleasing, discovering rats like the scent of peanut butter and vanilla. My mentor approved my proposal to examine the olfactory system in autistic rats.

Finding My Mentor

I met my mentor, autism research scientist, Patricia Whitaker, Ph.D., at the National Alliance for Autism Research (NAAR) 2005 Annual Kickoff Luncheon, where she received an award for her autism research. Her acceptance speech captivated me. I introduced myself and volunteered to assist in her laboratory. I was assigned to aid graduate students with their autism research. At that NAAR luncheon, I was serving as a table captain, having been a team captain for annual NAAR Walkathons since 6th grade. In seven years my team raised a cumulative total of \$35,000. In 2006, NAAR merged with much better capitalized Autism Speaks and subsequently Cure Autism Now (CAN) joined with Autism Speaks.

Time Management Advice

Assiduous study skills I developed as a young child were vital throughout my high school years. The key to implementing long term projects is avoiding procrastination. I schedule an extensive task's completion with a structured, logically sequenced time table. These guidelines enable the timely completion of assignments with minimal stress.

The summer preceding senior year, a busy schedule awaited me. Added to my coveted Intel STS research, were two lengthy, European novels for AP English, Rousseau's Social Contract for AP U.S. Government, SAT prep., college visits, Drivers Ed, varsity cross-country team training, shadowing my autistic brother at library craft classes, coaching young autistic distance runners, teaching and working out at karate classes, and a nine day Disney World vacation. Using my structured time management model, everything neatly culminated before summer's close. I had a very enjoyable summer.

I applied my structured time management strategy to my Intel STS research. Volunteering in my lab commenced in the summer before my high school junior year (2005), and continued through my junior year. I acquired vital, relevant, lab skills, before embarking on my project. Throughout June 2006, I comprehensively read many scholarly papers on human autism, animal models of autism, hypersensitivities, and the autistic brain. Gleaning knowledge about my topic and selecting my hypothesis. I then built all the testing devices my experiment required. My scientific procedures, data gathering, and computation of statistical significance were complete by the end of July, allowing ample time to write and edit my scientific paper.

Acquiring New Skills

My laboratory espouses the scientific attitude of collaboration and communication. Researchers share methods and results. Research grant applications include everyone's data and discoveries. Upon entering the lab in July 2005 as a volunteer, a graduate student took me under his wing, teaching me research methodology and analysis of data for significance (ANOVA and LSD Post Hoc tests). I learned to count behaviors on videos, use a tally system, and identify the significance of many behaviors. Scientific papers authored by my mentor and peer reviewed journals were my preliminary introduction to current knowledge. Three years of InSTAR, an independent research class in my high school, in grades 10, 11, and 12, taught me the skills to read scientific papers, understand research methods, compute statistics (t-test, z scores, chi squares, standard deviation, and standard error of the mean), and most importantly all the sections required in writing a thorough scientific research paper. My research project is built on previous scientists' accomplishments, bringing prior discoveries another tiny step closer to

solving autism's mysteries. The data I gathered in my autism study are included in the Stony Brook University lab's larger autism study submitted for publication and a new grant application.

Science and Imagination

An inquisitive individual is science's fuel. Imagine, if nobody speculated about novel ideas. Today's highly advanced technical world wouldn't exist without mankind's zealous desire to investigate and obtain knowledge. Modern societies couldn't have evolved if humans didn't wonder, "*What if...?*" True scientists must take the initiative to follow their curiosity. Energetic commencement and persistence drive scientists' acquisition of knowledge. Careful contemplation and evaluation are the initial steps facilitating discoveries. Science thrives on the initiative of probing individuals, following their pioneering ideas. Curiosity and initiative led me to discover a tiny missing piece in autism's puzzle, bringing mankind one step closer to finding a cause and cure. Had I not asked myself "*What if...*" the conundrum of autism would be one stride further from completion.

PART 2: THE RESEARCH

Key Facts About Autism

Autism is a pervasive developmental disorder with language, cognitive, and social impairments, often accompanied by hypersensitivity, or an over-reaction of one or more of the five senses. Autism is a spectrum disorder, meaning there is a widely varying severity of symptoms, and that no two autistic individuals are alike. Some key symptoms of autism include lack of creative play, repetitive body movement, ritualistic behaviors, minimal emotional

attachment to others, and tactile and auditory hypersensitivity. Autism's incidence is currently on the rise; 1 in every 150 children born is later diagnosed with autism.

Purpose

The occurrence of autism is 4 males: 1 female. However, more severe pathology is necessary to cause autism in females than in males. As a result, when a female has autism her symptoms are more severe. Research indicates that autistic females are more cognitively impaired than autistic males. But are autistic females more impaired in all excesses and deficits of the disorder, or only in specific areas? The purpose of my research is to determine whether autistic females experience greater hypersensitivity than their autistic male counterparts.

Importance of Study

Hypersensitivity plays a large role in everyday lifestyle. It interferes with attention span, ability to learn, and overall life quality. For example, a hypersensitive individual may be offended or distracted by the teacher's perfume and consequently unable to concentrate in class. Identifying these hypersensitivities can lead to removal or modification of the environment to improve learning and life comfort.

Methods

The most common finding in autistic individual is a 50-70% increase in blood platelet serotonin level, and a decrease in brain serotonin level due to the negative feedback response. This study uses a rat model of autism that mimics human autism. Behaviors of rats injected with 5-methoxytryptamine (5-MT), a serotonin agonist, were compared with behaviors of control rats

which were injected with saline. The increased serotonin levels from the 5-MT injections impair serotonin growth terminals, producing “autistic like” behaviors in the rats. This study examined 32 rats: 8 5-MT (autistic like) males, 8 5-MT females, 8 saline males, and 8 saline females. The testing took place when the rats’ age was comparable to a young adult human.

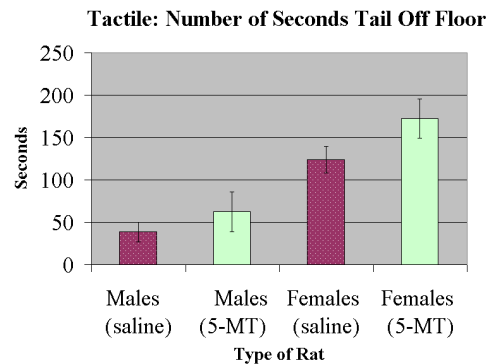
Statistical Tests

Statistical tests measured significance in the rat behaviors. First an ANOVA determined if there was any statistical significance present between any of the four rat groups. Then a LSD Post Hoc Test determined between which groups the statistical significance existed.

Tactile Analysis (Part 1)

The first section of my experiment was tactile analysis. To measure tactile hypersensitivity, the bottom of an open field was lined with 36 grade sandpaper, serving as the tactile stimulus. Each rat was to be placed in the open field for 5 minutes. All rat behaviors were video recorded, to be analyzed later with stop watches and clickers. Rats typically walk with their tail dragging on the floor behind them. The rat’s tail is a very sensitive

Figure 1:



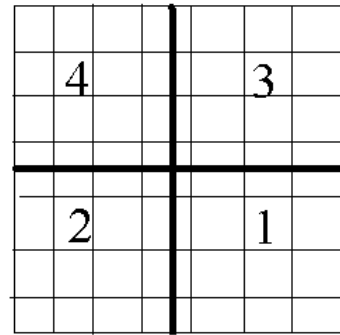
area. If the rat is hypersensitive it lifts its tail off the floor. As seen in **Figure 1**, the 5-MT (autistic like) females elevated their tails off the floor for significantly more time than the 5-MT males, suggesting the 5-MT females were significantly more hypersensitive to tactile stimuli

than 5-MT males. Additionally, the saline (control) females lifted their tails off the floor significantly longer than the saline males, indicating increased sensitivity in the control female.

Auditory Analysis (Part 2)

The second component of this study was auditory analysis. A beeping alarm clock, positioned just outside quadrant one of the open field (**Figure 2**) served as the auditory stimulus. For five minutes each rat was placed in the open field, and all behaviors were video recorded for further analysis. The number of grid lines each rat crossed during the five minute period was quantified to measure anxiety level in the rats.

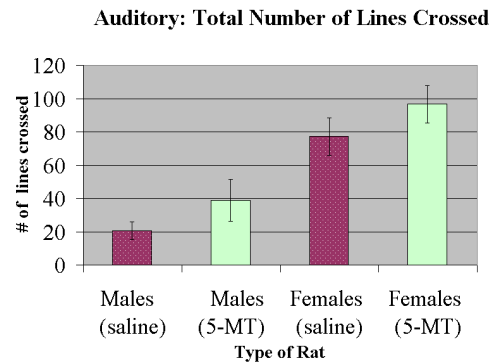
Figure 2:



Alarm

As seen in **Figure 3**, the 5-MT female rats crossed significantly more lines than the 5-MT males, showing increased anxiety and auditory hypersensitivity in the 5-MT females. The number of times each rat reared (stood on two hind legs) was measured as an indication of escape behavior, during the five minute period.

Figure 3:



When one of their hypersensitivities is triggered many autistic individuals will try to escape from the situation. Once again, the 5-MT females displayed significantly more rearing behavior than the 5-MT males (**Figure 4**). Additionally the saline females

Figure 4:

Rat Group	Auditory Test: Number of Times Rats Reared
	Mean ± Standard Error of the Mean
Male Saline	1.000±0.500
Male 5-MT	7.500±3.065
Female Saline	17.125±3.372
Female 5-MT	24.250±3.483

crossed significantly more lines (**Figure 3**) and reared significantly more (**Figure 4**) than the saline males, indicating an increased sensitivity state in the control females. The initial hypothesis was that the 5-MT males and females would spend significantly less time in quadrant 1 and more time in quadrant 4 (**Figure 2**), than the saline males and females. It was hypothesized hypersensitivity to the auditory stimulus would cause the “autistic like” rats to run as far away as possible from the offending stimulus. However this did not prove to be the case; there was no significance in amount of time any of the four rats groups spent in each of the four quadrants. During the five minute period, the 5-MT males froze in position, and the 5-MT females scurried around randomly and incessantly. The 5-MT male and 5-MT female rats both have autism, yet they reacted to the auditory stimulus with diametrically opposite behaviors. This observed reaction supports the theory that autism may be caused by different triggers in males vs. females, but both manifest the same class of symptoms, which define autism.

Olfactory Analysis (Part 3)

The third part of this study included an olfactory analysis of autistic rats’ behaviors. Many autistic individuals are extremely hypersensitive to olfactory stimuli, yet this sense has rarely been studied in the autistic population. Thus this section of the study serves as a baseline for further olfactory research.

Small amounts of two scents, which the typical rat finds pleasing, peanut butter and vanilla extract, were placed in enclosed containers in two separate corners of the open

Figure 5:

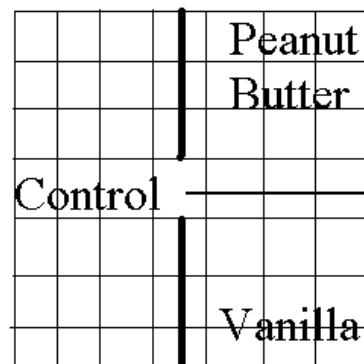
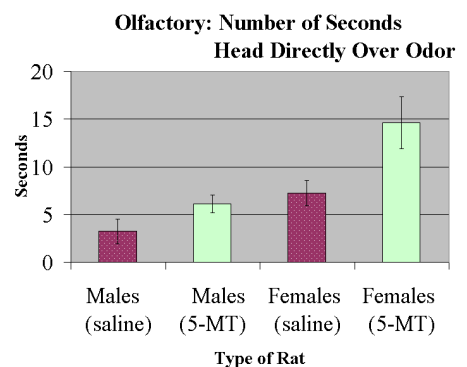


Figure 6:



field (**Figure 5**). It was hypothesized the “autistic like” rats would spend significantly more time in the control area, away from both the peanut butter and vanilla scents, due to autistic trait of hypersensitivity and consequent repulsion to odors. However, the “autistic like” rats spent more time with their noses directly over the peanut butter and vanilla scents than the control rats (**Figure 6**). And the 5-MT females spent significantly more time with their noses directly over both scents than the 5-MT males (**Figure 6**). Additionally the 5-MT males spent more time in the vanilla quadrant than the control males. Possibilities for these unexpected outcomes are that sniffing is a self stimulatory behavior, or the “autistic like” rats were hyposensitive, or distracted by these two scents causing their fixation on them resulting in continuous sniffing.

Conclusion

Hyperserotonemic (5-MT) female rats were more hypersensitive than the hyperserotonemic male rats to tactile, auditory, and olfactory stimuli. The hyperserotonemic female rats displayed exaggerated atypical symptoms of similarly diagnosed males. In other words, whatever atypical behavior the 5-MT male rat showed compared to the control male rat, the 5-MT female showed this behavior with increased severity compared with the 5-MT males. In all three senses studied (tactile, auditory, and olfactory) the control female showed increased sensitivity responses compared to the control male. Increased hypersensitivity in the female 5-MT rats compared to the male 5-MT rats is reflective of this.

Autistic individuals’ behavioral manifestations may be due to impairments in the cerebral cortex and limbic system. Both show abnormal development in autistic individuals. Future research could pursue post mortem studies, correlating typical neurons in the auditory cortex, sensory cortex and olfactory bulb with impaired neurons in the hyperserotonemic rat model of

autism. Study of the human genome is needed to find genetic mechanisms that cause autism and its behavioral manifestations.