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Decreases in the Frontal Cortical Areas Following a Developmental Disruption Model of Schizophrenia

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Abstract

Methylazoxmethanol Acetate (MAM) is a toxin that temporarily blocks mitosis in developing embryonic brains. Exposure in rats on embryonic day 17 (E17) selectively targets frontal and hippocampal regions of the brain and produces behavioral and anatomical effects strikingly similar to those seen in human patients with schizophrenia. While previous studies examining these induced neuroanatomical disruptions support E17 MAM exposure as an animal model of schizophrenia, the vast majority focused on male rats. However, there have been a dearth of studies specifically looking at female rats in this model. This is significant since there is evidence of sex differences in the onset and symptomologies in humans with schizophrenia. The current study utilized volumetric analysis and point counting on pre-sliced rat brain tissue to determine anatomical differences in the size and overall volume of the frontal cortical areas in female rats exposed to the MAM toxin on E17. We found significant decreases in the brain weights ($p < .05$), body weights ($p < .05$), and volume of the frontal cortical areas ($p < .05$) in female rats exposed to MAM on E17. These results indicate that the MAM group expressed lower brain volume in the frontal cortical areas than the saline-treated control group. In future studies, this data can be compared to male rats to examine sex differences in MAM exposure.

Keywords: schizophrenia, MAM model, frontal cortical areas, developmental disruption

Decreases in the Frontal Cortical Areas Following a Developmental Disruption Model of Schizophrenia

Schizophrenia is a psychiatric disorder that encompasses symptoms such as hallucinations, cognitive dysfunction, and delusions that often lead to a lifelong impairment of certain brain functions (Volk & Lewis, 2015). This disorder afflicts around 21 million people worldwide, and these patients can experience a wide range of positive and negative symptoms (Nuño et al., 2019). Within the symptom set for schizophrenia, perhaps one of the most prominent and consistent symptoms is a level of cognitive dysfunction. Krzystanek et al. (2011) found that around 70 percent of schizophrenia patients studied were found to have cognitive dysfunctions likely relating to their disorder. These dysfunctions are likely related to decreased activity in the prefrontal cortex (PFC) and frontal cortical regions. This decreased activity is thought to manifest as cognitive deficits, such as deterioration of attention, operative memory, learning, and executive functions (Krzystanek et al., 2011). Because of this, cognitive deficits can be a key warning sign of someone beginning to express schizophrenic symptoms.

The causes of schizophrenia are still heavily debated, but it is likely that multiple factors are involved (Haller et al., 2014). Past research has concluded that excessive dopamine may contribute to schizophrenia onset, however, it is now becoming clear that other neurotransmitters such as GABA and glutamate are also factors at play (Haller et al., 2014). Epigenetics is also a factor, meaning that environmental factors can interact with susceptibility genes which can increase the risk of schizophrenia (Haller et al., 2014). Considering these findings, it can be

concluded that a multidisciplinary approach and individualized care are essential when treating schizophrenia (Nuño et al., 2019).

Methylazoxmethanol Acetate (MAM) and the MAM Model

Methylazoxmethanol Acetate (MAM) is a toxin that inhibits the mitosis of currently dividing neuronal cells in the prenatal brain of an organism (Lodge & Grace, 2009). The rapid division of cells in an organism's brain in the prenatal stage is crucial to a healthy and normal development of the fetus. When MAM is introduced on embryonic (E) day 17, it causes a disruption in mitosis and selectively targets brain structures that have been shown to have abnormalities in humans with schizophrenia (Hradetzy et al., 2012). It can have detrimental effects on areas of the brain such as the hippocampus, corpus callosum, frontal regions, along with others (Lodge & Grace, 2009). These areas mainly include those that control personality, behavior, and memory. These abnormalities mirror some of the aspects of schizophrenia behaviorally and anatomically, which is why this agent is used as a neurodevelopmental model for the disorder (Hradetzy et al., 2012).

Animal models such as the MAM model enable researchers to study the underlying mechanisms of cognitive disorders in the brain that are not possible to emulate in humans (Lodge & Grace, 2009). Through these mechanisms, anatomical abnormalities in the structure of rat's brains have been found which have been consistent with the brain areas that are most closely related to schizophrenia symptomologies, such as the hypothalamus, corpus callosum, and hippocampus (Hradetzky et al., 2011; Huo et al., 2018; Perez et al., 2019). For example, through analysis of 10 control rats and 10 MAM treated rats, it was found that not only were there alterations to the hippocampal and frontal cortical regions, but there were also alterations in

specific receptors such as AMPA in MAM-treated rats (Hradetzky et al., 2011). These results suggest that MAM disrupts the anatomical structures of the rats' brain as well as the communication networks and receptors responsible for typical brain function. The MAM model allows us to research schizophrenia in a way that could not be done in humans. Due to the similarities between human and rat brains (Dalley et al. 2004; Kesner & Churchwell, 2011), we can better understand the human brain and the underlying mechanisms of schizophrenia. If the MAM model and its implications are studied enough, there could be real pharmaceutical advancements made for humans diagnosed with schizophrenia.

Developmental Origin of Schizophrenia

The pathogenesis of schizophrenia is dependent on multiple factors, but it has been supported through previous research that schizophrenia may have a developmental origin. The neurodevelopmental hypothesis of schizophrenia states that events during fetal gestational growth disrupt the course of normal brain development, and in turn creates vulnerabilities that can cause predispositions for the development of schizophrenia later in life (Rehn & Rees, 2005). Perhaps the most supportive evidence for this hypothesis comes from the concept of hippocampal dysplasia (Kovelman & Scheibel, 1984). A recent study conducted by Roeske et al. (2021) concluded that incomplete hippocampal inversions (IHI) were more prevalent in schizophrenia patients in both the right and left hemispheres, which can also lead to volume reductions in the brain. This is significant because the only time that IHI can happen is during prenatal development, suggesting that brain abnormalities in schizophrenia have a developmental origin and can cause a predisposition for the disorder as a result (Roeske et al., 2021). Findings from Davis et al. (1995) also support this hypothesis. This research focused on monozygotic (MZ) twins, or twins that come from the same egg, in hopes to determine the influence of

prenatal development and its impact on the morphology of schizophrenia later in life (Davis et al., 1995). It was found that MZ twins who had shared the same blood supply and placenta had a 60 percent concordance rate in comparison to 10.7 percent for twins who did not share the same placenta and blood supply (Davis et al., 1995). Because twins who share the same blood supply are likely to share infections and developmental abnormalities, data from Davis et al. (1995) supports the neurodevelopmental hypothesis of schizophrenia.

Previous research has found that instances of intrauterine malnutrition, short gestation (< 37), and low birth weights are associated with an increased lifetime risk of developing schizophrenia along with other disorders (Jones et al., 1998; Wahlbeck et al., 2001). The phenomena of preeclampsia, or the reduced nutritional supply to fetus in utero, is a complication that has been found to be most strongly associated with schizophrenia later in life. Therefore, creating an optimal prenatal environment for a developing fetus is critical to avoid predispositions to the development of schizophrenia and other cognitive disorders. Wahlbeck et al. (2001) concluded after analyzing a cohort of 7,086 participants born at Helsinki University Central Hospital in Finland that both males and females who were born with low birth weight and short body length had increased risk of schizophrenia in human patients. This study also found that children with small birth length and who were below the lowest BMI percentile at age seven were four times more at risk of developing schizophrenia (Wahlbeck et al., 2001). Since the factors of low birth weights and low BMI throughout development can be a predictor of schizophrenia development later in life, there is importance in analyzing these factors while conducting the current study.

Sex Differences: Why Female Rats?

In completing sex-specific research, it is important to make the distinction between gender and sex. Sex, a complex variable in research, refers to the distinctions between “male” and “female” on the basis of biological differences (Tseng, 2008). Gender is a combination of socio-cultural processes and attitudes that shape “masculine” and “feminine” behaviors and identity (Klein et al., 2015). When referring to the male and female sexes in both rats and humans, we are specifically referring to the biological basis of the female sex and not the gender identity or expression of human individuals.

There are observable sex differences in human males and females that experience schizophrenia, however, most studies using the MAM developmental disruption model to study the disorder have focused on comparing these differences in male rodent brains (Perez et al., 2019). Due to this disparity, few studies have examined the differences specifically in female rats. There is ample evidence to suggest that there is significant symptom onset of schizophrenia during menopause, meaning that differences between males and females are an important aspect of research (Salem et. al., 1998). As previously mentioned, males are observed to have a symptom onset that occurs mainly in their 20s with a drop in these onsets after their first admission (Riecher-Rössler et al., 2018). However, in females, we see the opposite effect, in which symptom onset seems to be more likely once they reach their 40s (Salem et. al., 1998). It has been hypothesized that these changes are due to the estrus cycle, which lines up with this fact that female symptom onset tends to peak during menopause in their mid-late 40s (Hill & Fitch, 2011).

The estrogen-protective hypothesis of schizophrenia has been developed based on observations in which schizophrenia onset and symptomology have been affected based on an individual’s stage in the estrus cycle (Hill & Fitch, 2011). Broadly, it has been observed that a

drop in estrogen exacerbates symptoms of schizophrenia such as psychosis, whereas periods of high estrogen improve symptoms and/or protect the female sex from symptom relapse (Perez et al., 2019). Estrogen has been observed to protect cells against inflammation and apoptosis and there is even evidence to suggest that estrogens can improve synaptic plasticity, making such findings about the neuroprotection of estrogen unsurprising (Perez et al., 2019). Furthermore, a crucial factor in the differences between a male versus female neonatal brain is sex differences in androgen levels, which typically lead to impacts on brain morphology (Hill & Fitch, 2011). With these factors in mind, studying sex differences in schizophrenia is critical to better understanding the disorder in a more sex-specific way.

There are a few possible explanations as to why there is a difference in symptomology and onset between sexes that may not have to do with hormonal changes in humans. It has been found that males tend to experience more negative symptoms, such as social withdrawal and lack of motivation, while females tend to exhibit more positive symptoms, such as paranoia (Riecher-Rössler et al., 2018). Riecher-Rössler et al. (2018) found that these sex differences in symptomologies could be attributed to how certain activities or cognitive processes can be comorbidities for schizophrenia. For example, males are more likely to experience substance or alcohol abuse earlier in their lives, which is a risk factor for developing schizophrenia, as well as deficits in communication and social ability. On the other hand, females tend to participate less in substance abuse earlier in life and have been found to outperform males in emotional recognition and regulation and verbal learning tasks (Riecher-Rössler et al., 2018). These differences in comorbid activities and cognitive performances could partly explain why symptomology is different between the sexes.

Research on sex differences in schizophrenia symptom onset between males and females is somewhat inconsistent, with disagreements between which sex has the most prevalence of schizophrenic symptoms. This is likely attributed to the underrepresentation of the female sex in schizophrenia research, as well as the trend of diagnosing female patients with schizoaffective disorder instead of schizophrenia or schizophrenia-related psychosis (Riecher-Rössler et al., 2018). In any case, it is clear that the demand for female-centered studies on schizophrenia are necessary.

The MAM developmental disruption model of schizophrenia has consistently shown sex differences between male and female rats in MAM-treated groups. As previously mentioned, this spike is likely due to the changes in hormone levels such as estrogen and progesterone. There is also evidence to suggest that the stage of the estrous cycle a female is experiencing can be dependent on how the augmented dopamine system functions in the brain (Perez et al., 2019). As previously mentioned, dopamine regulation plays a key role in the severity and presence of schizophrenic symptoms. Therefore, the regulation of these systems is a key aspect of schizophrenia research in rodents. However, it has been shown that many studies do not account for the changes in human and rodent estrous cycles even though they are being found to influence behavior and neuronal activity (Perez et al., 2019). These hormonal changes could not only explain the differences we see in rats in the MAM model, but they could also be used as a tool to explain the symptom onset and severity in female patients with schizophrenia. Perez et al. (2019) report that the ventral tegmental area (VTA) increases in activity during estrus, as well as findings suggesting that dopamine neuron population driven by progesterone signaling in the ventral hippocampus drives hyperactivity of these neurons in female rats. Because estrogen and the estrous cycle have been previously shown to affect specific areas of the brain only in female

rats, it is important to understand how these differences may manifest in other brain areas as well.

Hypofrontality and the Frontal Cortical Areas

The frontal cortical areas of the brain have been shown to exhibit hypofrontality, or the decrease in activation due to a decreased cerebral blood flow to this area in humans with schizophrenia (Cummings et al., 2021). Hypofrontality has been documented to affect around 70 percent of patients with schizophrenia (Krzystanek et al., 2011). Such consequences of hypofrontality include longer reaction times and less accurate working memory performance (Potkin et al., 2009). Due to the prevalence of hypofrontality and similar dysfunctions which have the ability to deteriorate areas of the brain, research consistently finds that brains in humans diagnosed with schizophrenia weigh less when compared to healthy brains (Krzystanek et al., 2011). The presence of hypofrontality implies a link between frontal cortical dysfunction and the diagnosis of schizophrenia, meaning that it is crucial to study this brain region to better understand the impact of schizophrenia on the brain. This is especially true since the anatomical abnormalities found in patients with schizophrenia can manifest as cognitive dysfunctions that can disrupt and severely impact a patient's life, such as difficulty in short-term memory and deterioration of attention (Krzystanek et al., 2011).

The MAM developmental disruption animal model can mimic brain abnormalities present in human patients diagnosed with schizophrenia, and previous research has found these PFC deficits in rats previously used in MAM model testing (Chalkiadaki et al., 2019; Huo et al., 2018). Past research is inconsistent on the terms used to describe these areas, and what exactly the PFC includes as it is not as defined of an area in rats as it is in humans. Laubach et al. (2018) conducted a meta-analysis of research on rat PFC and related frontal cortical regions in hopes of

clarifying exactly what regions of the brain are PFC. The researchers concluded that to be transparent about research, the name given to the area of study is not as important as specifying exactly what structures are being analyzed and at or between which bregma (Laubach et al., 2018). Bregma refers to the marker used to determine where in the brain tissue is located on a stereotaxic atlas.

For the present study, we did not use the term PFC, rather we decided the term frontal cortical areas was more inclusive of the structures we wanted to study. The frontal cortical areas are the name given to the grouping of cortical regions that control social behaviors, movement, executive functioning tasks, and somatosensory functions in a rats' brain. This area has been chosen for this study because of the similarities in these structures to each other and their relation to the function of the frontal cortex in humans with schizophrenia. These areas were chosen also to simplify the point counting process that was used to gather data, as well as make sure estimation was more consistent. The areas we will be measuring are the frontal association area, primary and secondary motor cortex, orbital cortex, prelimbic cortex, cingulate cortex, angular insular cortex, infralimbic cortex, primary somatosensory cortex, dorsal peduncular cortex, granular insular, and the dysgranular insular cortex. These frontal cortical areas fall between bregma 6.12mm and 2.52mm.

The structures of frontal cortical regions in rodents have a general analogous structure with the PFC in humans. Because of this, it is possible to make some comparisons between these two structures in two different species. Kesner & Churchwell (2011), after a review of the PFC in rats compared to humans, found data to suggest that there is sub-regional specificity within the PFC of rats and humans as well as parallel cognitive functions of the different subregions of the PFC. Although rodent brains do not contain the complexity comparable to that of a human, they

still provide structural and functional similarities that allow rodent models to be useful research tools (Kesner & Churchwell, 2011). The PFC across species may not necessarily be compared entirely through exact anatomical similarity; rather, they are considered analogous structures if they are responsible for the same facets of functioning. More specifically, the PFC encompasses the areas listed previously for the present study because of the connections to the thalamocortical regions towards the midbrain in both rodents and humans (Dalley et al., 2004). This is paired with the fact that the PFC in any species receives extensive inputs from the posterior parietal cortex and sensory cortical areas (Dalley et al., 2004). As laid out in Dalley et. al. (2004), the cortex areas we will be examining for the current study are analogous to the criteria met for the PFC in other species, including humans. In the MAM model for schizophrenia, these similarities can allow us to draw comparisons between the rat and human frontal cortical areas to better understand how schizophrenia affects this brain structure.

The Present Study

Schizophrenia is a multidomain disorder that may have developmental origins beginning in the prenatal period. The MAM model can indicate which aspects of development are affected by the MAM toxin, and in turn, give us insights into the developmental impacts on human patients with schizophrenia. When administered on E17, the MAM toxin specifically targets frontal lobe structures, which contribute to several cognitive dysfunctions. There is a dearth of research focusing on female rats in the MAM model, while studies with male rodents are more commonly conducted. In human patients with schizophrenia, sex differences are clear in symptomology and onset. To accurately make comparisons between male and female rats in the MAM model, as well as understand sex differences in schizophrenia, studying female rats is crucial.

The main analysis of the present study aimed to examine the volume differences in the frontal cortical areas of female rats treated with MAM in comparison to control rats who were treated with a placebo. We are predicting that the MAM-treated female rats will have a decreased volume in these frontal cortical areas in comparison to the control group. The present study aimed to give insight into what areas of the brain are affected by MAM, as well as validating the MAM model as a neuropathological model for schizophrenia in female rats. Frontal cortical area volume will be measured using systematic sampling in the stereological estimation model (Gundersen et al., 1987). This method utilizes the technique of point counting, which was completed utilizing the ImageJ computer program. The frontal cortical areas of the pre-sectioned rat brains were studied. The present study utilized a small sample of brain tissue collected by Penley et al. (2016). While this study looked at behavioral effects of the MAM model on rats through memory tasks, the present study will focus on the anatomical differences in tissue from these animals after they were sacrificed. If there is a significant anatomical difference in the volumes of the control versus experimental rats in the frontal cortical areas, we may be able to conclude that MAM has targeted these areas and they are affected by the toxin. To contextualize our results, additional analysis on body weights and brain weights of the rats were run. We are predicting that the female rats will have reductions in both body weight and overall brain weight due to the effects of the MAM toxin. In completing these analyses, we aimed to gain insight into the developmental origins of schizophrenia as well as how specifically the brains and bodies of these animals are affected by the MAM toxin.

Methodology

Animals and MAM exposure

All past experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) (Protocol #95-2016, Wheaton College) and the assurance for the humane care and use of teaching and research animals at Wheaton College (Penley, 2016). In this study, Sprague Dawley rats, ranging in age from birth to adult animals were used. Five pregnant dams underwent MAM injections (25mg/kg, i.p.) on gestational day 17 and four control dams received physiological saline (1ml/kg, i.p.) on this same day (Babcock et al., 2016). The rats used for this study were exposed in utero at this time. After litters were born, they were separated into litters of 10 with five females and five males in each group on postnatal (P) day 1 (Babcock et al., 2016). At P21, the rats were weaned, tagged, and housed in pairs in laboratory cages with a 14:10 hour light/dark cycle with access to unlimited food (Babcock et al., 2016). From this sample, we analyzed six control females and seven MAM females in the present study. We originally started with a sample size of 19 rats, but some had to be excluded from the study because of damaged or missing tissue. In the end, it was concluded that 13 rats had enough viable tissue to analyze properly and accurately.

Behavioral Tests and Weighing Before Histology

These animals had previous behavioral testing through a water maze structure. This maze was an eight-arm radial water maze, which was designed to test the working memory of the control and MAM-treated rats (Penley et al., 2016). After this testing, it was concluded that female rats who were treated with the MAM toxin were more likely to show working memory differences, which has led us to believe that there are differences in the frontal cortical areas (Penley et al., 2016). Beginning at P21, rats were weighed consistently at the same time and day each week until P95. After weights were taken and the rats completed the behavior water maze

test, they were euthanized and their brains were sliced for histological analysis (Penley et al., 2016).

Brain Tissue

The rat brains were sectioned in the coronal plane at 50 microns using the cryostat (Babcock et al., 2016). Animals were perfused at P95 using transcranial perfusion (Babcock et al., 2016). The reason for this perfusion was to make sure that the tissue stayed intact during the rest of the staining process, as to preserve the tissue in as much of a life-like state as possible. Following this, the transcranial perfused brains were removed and placed in a four percent formaldehyde bath (Babcock et al., 2016). The tissue was then left to sit in a 10 percent sucrose bath for two weeks and after this, the tissue was moved to a 30 percent sucrose solution for another two weeks (Babcock et al., 2016). The purpose of this process is to decrease the likelihood of tissue getting damaged. The tissue was sliced at 24-27 degrees Celsius (Babcock et al., 2016). The tissue was mounted and stained using Thionin, a chemical staining agent that creates a clear vision of structure and cell bodies (Babcock et al., 2016).

ImageJ and Point Counting

Images of the rat brain tissue were taken on a 2x Nixon E-200 microscope. We used stereological estimation and point counting to determine the volume of the frontal cortical areas on the rat brain tissue (Gundersen et al., 1987). Stereological estimation involves calculating the area and volume of a given sample (West et al., 1991). We can use this data to determine the overall size of the brain area that we have selected, which is gathered through the process of point counting. This technique was used to count the number of cross-sectioned lines of the overlayed grid that fall within a brain structure that was chosen to analyze (See Table 1 in Appendix). Point counting is useful for estimating the volume of tissue sets and it is also useful

in reducing the inevitable variability in the area estimation process. Point counting is completed by individually uploading the desired images of rat brain tissue to the ImageJ software, which was accessible via download on a personal computer. All images were uploaded to a Google drive for easy access and organization. A 0.11 mm^2 grid overlay was applied to the image using ImageJ, which is then used to measure the volume of the desired brain structure (See Figure 1). This grid is then utilized by counting the cross-sections in between the grid within the desired brain structure (See Figure 1). Once the area was counted, the data containing the number of points counted on each tissue slide was collected and recorded in an Excel spreadsheet. For this study, the frontal cortical areas in rats were measured. This point counting method can also be useful in tracking cellular disarray in the brain in each tissue sample, which is a common aspect of individuals experiencing schizophrenic symptoms (Threlkeld et al., 2007). Volumetric analysis was computed first through Cavalieri's estimator of volume, which then gave us the area of each rat's frontal cortical area structures. As previously mentioned, point counting was used to gather data on rat brain tissues, specifically volumetric information about the frontal cortical areas. This allowed us to compare MAM-treated frontal cortical areas to the control group.

Data Analysis

The area of each frontal cortical region in each rat were measured using ImageJ. The total volume of all frontal cortical areas computed was compared between control and MAM groups using an independent samples *t*-test. This is necessary to the current study because we will need to compare and test the statistical significance of means of frontal cortical area volume between the MAM-treated group and the placebo-treated control group. Volumetric measures were compared across the female treatment groups of MAM-treated versus control rats. If the control group is found to have a significantly larger volume, then we can conclude that MAM has

affected the size of the animals' frontal cortical area. However, we cannot say whether this size difference is due to white matter loss or changes in cell density. The SPSS software was used to analyze this data to determine if there is a significant difference in the size of the MAM-treated rats versus the control rats.

To contextualize our main finding, additional analyses on body weight and brain weight were conducted. A repeated-measures ANOVA test with between-subjects comparison was conducted to determine if the rats in each group gained weight in the same way across the weighing periods of P21-P95. For post hoc analysis, independent samples *t*-tests were used to examine differences between the two groups at each day. Using an independent samples *t*-test, the overall brain weights of rats were also analyzed. Because of the phenomena of hypofrontality and its effects on brain weights in human schizophrenia patients, it is important to study these variables in the MAM developmental disruption model in the comparable frontal cortical areas as well. We used an alpha value of .05 for all statistical testing. Cohen's *d* was used to determine the size of the effect when comparing the two groups.

Results

The present study's main analysis looked at a MAM model of disruptions' impacts on the frontal cortical areas. Studying the impact of MAM is important in understanding the brain changes we see in schizophrenia, the disorder which this disruption model emulates in rodents. This study had a sample size of $N = 13$ rats, with $N = 6$ control rats and $N = 7$ MAM rats. We examined body weight, overall brain weight, and the volume of the frontal cortical areas between groups.

Body Weight Analysis

We first examined the body weights of all of the MAM and control rats that were used in the present study. To compare the differences of these rats' overall brain weights, we looked at the days in which the rats were weighed originally before they were sacrificed for histology in Penley et al. (2016). Using archived data from this previous study, the rats were tracked beginning at P21 and weighed once each week for 12 weeks until P95. A repeated-measures ANOVA with a between-subjects design was used to measure the weights of rats between P21 and P95. We looked at the main effects of "day" to track weights across the study, and the main effect of "condition" to examine the impact of MAM on body weights. We also examined the day-by-condition interaction, which allowed us to determine if there were different weight gains between the groups over time.

The weight was tracked between weaning (P21) through adulthood (P95). We found a main effect of day, $F(11, 121) = 403.9$, $p < .05$, in our between-subjects comparison. The results suggest that the rats grew steadily between P21 and P95 in both groups, which indicates typical growth over days. We then found a main effect of condition in the between-subjects comparison, $F(11, 121) = 61.22$, $p < .05$. This indicates that the two groups were significantly different from each other in terms of their body weights. Finally, we found a day-by-condition interaction, $F(11, 121) = 3.74$, $p < .05$. This indicates that the rats were not increasing in weight in the same ways. Because we found main effects of condition, day, and the interaction between the two groups, we conducted an independent samples *t*-test to compare each of these days. We found that all *t*-tests comparing days to the MAM and control conditions yielded significant results, with all outcomes having a $p < .05$ (See Figure 2). At the conclusion of the study (P95) the rats were weighed to determine the final group differences. The average weight of the control group ($N = 6$) was calculated to be 262 grams ($SD = 11.44$). The average weight of the MAM group (*N*

= 7) was calculated to be 217 grams (*SD* = 14.67). On P95, we observed a 17% decrease in body weights of the MAM group when compared to the control group. After utilizing an independent samples *t*-test, we found a significant decrease in brain weight in the MAM groups, $t(11) = 6.081$, $p < .05$ (See Figure 3). A Cohen's *d* also revealed a very large effect size for body weights at P95, with $d = 3.42$.

Brain Weight Analysis

MAM model research has consistently found that brain weights of MAM-treated animals are significantly lower throughout development, which mirrors research in schizophrenia that states lower brain weights are a premorbidity for the disorder (Krzystanek et al., 2011). To determine if brain weights were significantly different between conditions, we ran an independent samples *t*-test. The control group (*M* = 1.83 grams, *SD* = 0.08) and the MAM group (*M* = 1.58 grams, *SD* = 0.06) were compared in overall brain weights. We found a significant difference between the MAM and control groups in terms of final brain weights, $t(11) = 6.635$, $p < .05$ (see Figure 4). Specifically, the MAM group showed a 14% decrease in overall brain volume in comparison to the control group. These findings support our prediction that brain weights of MAM rats would be lower than the brain weights of the control group. Utilizing Cohen's *d*, we calculated the size of the effect between the MAM and control groups in terms of total brain weight. The effect size, which measures the magnitude of this effect for this study, was found to be a $d = 3.54$. This indicates that the effect between groups was large in impact.

Frontal Cortical Area Analysis

To find out if there was a significant difference specifically between MAM frontal cortical area volumes and control group frontal cortical areas, volumes were calculated using Cavalieri's estimator of volume. The average brain volume in the control group, *N* = 6, was

found to be 91.56 mm^3 (*SD* = 3.07) and in the MAM group, *N* = 7, the average brain volume was found to be 76.94 mm^3 (*SD* = 5.16). Utilizing an independent samples *t*-test, we found a significant difference in the frontal cortical area volume, $t(11) = 6.081$, $p < .05$ (See Figure 5). These results indicated that there is a significant decrease in brain volume between the control group and the MAM-treated experimental group, in which the MAM group expressed lower brain volume in the frontal cortical areas than the placebo-treated control group. Therefore, we can conclude that the MAM and control group had a significant decrease in the volumes of this brain area. Between the MAM and control groups, we also found that the MAM group had a 16% reduction in brain volume overall when compared to the control group. Further, we found a very large effect of MAM treatment. Utilizing Cohens *d*, we were able to calculate the size of the effect between MAM and control as $d = 3.38$. Because of this difference in volumes and the large effect size, we can conclude that the MAM rats have much lower frontal cortical area volume than the control rats. We can also predict that because of the large effect size, a larger sample size would still have found similar results. The data supports our hypothesis of MAM rats having significantly smaller frontal cortical areas.

Discussion

The current study aimed to examine anatomical differences in the size and overall volume of the frontal cortical areas in female rats exposed to the MAM toxin on E17. We predicted that the volumes in the frontal cortical areas of the MAM-exposed rats would be smaller than the control group because of the anatomical effects that MAM typically has on dividing neuronal cells during prenatal development. To better understand the effects of the MAM toxin on prenatal development and contextualize the main analysis of the current study, several additional analyses were run. The first analysis run was to determine the impact of MAM

on weight over time beginning at weaning (P21). We observed that MAM rats weighed less and did not gain weight in the same way over time. We also examined the brain weights of each rat to allow for a global estimate of brain changes following MAM treatment. Brain weight taken at the study endpoint (P95) showed that the MAM group saw a 14% decrease in overall brain volume. Finally, after having found large-scale changes in body weight and brain weight, we analyzed the frontal cortical areas of the rats to determine the impact of MAM on structures that are related to behavioral deficits found in humans with schizophrenia. We found that there was a significant decrease in frontal cortical volume by 16% in the MAM-treated group.

Brian and Body Weight Significance

Previous research has found that patients with schizophrenia often exhibit lower brain weights and decreased body weights throughout development compared to healthy individuals (Ellison-Wright et al., 2008; Hulshoff Pol et al., 2004; Jones et al., 1998; Wahlbeck et al., 2001). The reasons for these lower brain weights are multifaceted. Deficits in gray matter in the frontal lobe, cingulate cortex, and insular cortex have been found to be the reason for decreased brain weights in patients with schizophrenia (Ellison-Wright et al., 2008). The current study has analyzed these areas of the brain in both MAM-treated and control rats in the grouping of the frontal cortical areas as well as others. Since the MAM model is able to mimic the impacts of schizophrenia in human brains, it was not surprising to find that the brain weights of the rats analyzed for this study mirrored the phenomenon of lower brain weights in the MAM-treated group. It is predicted that metabolic abnormalities related to hypofrontality are due to the failure of normal connectivity between different brain regions (Cumming et al., 2021). The findings suggest that abnormalities exhibited in hypofrontality have the potential to cause connectivity

issues in different brain areas, which emphasizes why this phenomenon is important to study in the frontal cortical areas specifically.

We had originally predicted that the MAM-treated group would have a smaller brain weight based partly on impacts in human brains with schizophrenia. Neuroimaging studies have consistently shown metabolic abnormalities in the frontal lobe, specifically that there is decreased blood flow to this area in patients with schizophrenia (Knyazeva et al., 2008). Decreased blood flow to the frontal lobe can cause a multitude of effects both while this area is activated or not activated, which signifies that hypofrontality is not just activated when there is a deficit in completion of a cognitive task (Cumming et al., 2021). This indicates that hypofrontality is a lasting abnormality in patients with schizophrenia that can also have impacts on overall brain weight in humans with schizophrenia.

Dysfunctions such as hypofrontality can have impacts on brain weight because of decreased blood flow, which can also result in a progressive loss of cerebral grey and white matter in the brain (Van Haren et al., 2012). This progressive decrease has been found to affect a plethora of brain areas, such as grey matter decreases in the thalamus and hippocampus and white matter decrease in the corpus callosum (Van Haren et al., 2012, Hulshoff Pol et al., 2004). A meta-analysis of schizophrenia subjects found that overall, grey matter decreases have been reported at approximately 2% in patients with schizophrenia compared to healthy patients (Ellison-Wright et al., 2008). Hulshoff Pol et al. (2004) also found that symptom severity was correlated with a lower density of the corpus callosum, and this area along with others previously mentioned also seem to be affected depending on the severity of illness and symptoms. These abnormalities suggest that there is a greater issue at play, likely having to do with the neuronal networks of grey and white matter as well as the interconnected abnormalities of the corpus

callosum which is vital for hemispheric connections (Hulshoff Pol et al., 2004). Our findings suggested that there was a significant difference in the brain weights of MAM-treated rats compared to saline-treated rats. Because we used the final brain weights of these rats after weeks of development, it is fair to speculate that administration of MAM could be responsible for these observed brain weight differences. This difference in brain weight would also support the validity of the MAM model in which we see the mimicked effects of humans with schizophrenia on rat brains.

As previously emphasized, patients also exhibit lower body weights, which has been found to be a premorbidity for schizophrenia. Research has found that both human males and females who were born with low birth weight and short body length had an increased risk of schizophrenia (Wahlbeck et al., 2001). It has also been found that children with small birth length and those below the lowest BMI percentile at age seven were four times more at risk of developing schizophrenia later in life (Wahlbeck et al., 2001). This suggests that the risk of schizophrenia increases in prenatal development and throughout the first few years of human development. The current study saw these trends over the development of female rats in the MAM model as well, in which rats in the MAM-treated group did not gain weight in the same way over each weighing period through P21-P95. The MAM-treated group also saw a significant decrease in body weight when compared to the control group over each week of development.

Other studies have shown weight differences in hypoxia-ischemia (HI) patients, another developmental risk that mirrors the cognitive deficits in schizophrenia. HI occurs when there is decreased blood and/or oxygen flow to the brain, which can cause brain damage and adverse outcomes (Smith et al., 2013). HI can manifest in a few different ways, including tissue loss in white and gray matter of the brain (Smith et al., 2013). HI, usually occurring in children with

very low birth weight (VLBW) is considered a long-term neurologic morbidity in terms of cognitive dysfunctions (Hill & Fitch, 2011). These children with lower birth weights, according to previous research, have a greater risk of developing schizophrenia or other disorders in the future (Davis et al., 1995). The vulnerability of children born with VLBW and low brain weight causes them to be more susceptible to cognitive and behavioral deficits that affect attention, learning, and memory (Smith et al., 2013). These cognitive deficits signify that VLBW, as well as low brain weights, can be a predictor of cognitive abnormalities and disorders later in life. (Smith et al., 2013). This is precisely why it is important to take body and brain weights into consideration when conducting research on schizophrenia, especially considering a major symptom and indicator of the disorder is hypofrontality and lower brain weights which signify cognitive dysfunctions. Our results show that the MAM-treated animals exhibited a significantly lower body and brain weight when compared to the control group, which mirrors the consistencies found in humans diagnosed with schizophrenia. Since the anatomical phenomena of hypofrontality have been shown to have wide-reaching effects on the brain's function, we can confidently say that histological analysis is a crucial step in determining cognitive deficits and abnormalities in both MAM-17 animals and human schizophrenia patients.

Frontal Cortical Abnormalities and Sex Differences

It has been established that our findings show lower brain weights in MAM-treated animals than in our control group which is constant with previous research. Now, we must examine abnormalities specifically in the frontal cortical areas that may have played a role in the reduction of these brain weights. To reiterate, we found a significant difference between the volume in the frontal cortical areas between MAM-treated group and the saline-treated group with a large effect size. It is known that the PFC and associated cortexes are responsible for

facilitating executive functioning in rats, such as planning, rule-learning, and decision making. (Kesner & Churchwell, 2011). Because this area has an analogous function with the frontal lobe in humans, results of histological and behavioral outcomes using the MAM model can be used to help us understand abnormalities in human patients with schizophrenia. Through previous research, it has been found that the PFC and associated cortex areas are specifically affected by the MAM toxin.

Abnormalities in the prefrontal cortical histology in mice were found by Huo et al. (2018). Interestingly, these abnormalities were only specific to female mice. Huo et al. (2018) found that MAM-exposed female mice had smaller PFC and enlarged lateral ventricles with an 11% reduction in overall brain weight. Similar reductions have been observed in human patients with schizophrenia, meaning that MAM-exposed mice can model aspects of cognition such as spontaneous locomotion hyperactivity, memory deficits, impaired prepulse inhibition (PPI), and social recognition (Huo et al., 2018). This is significant when considering that the model for male mice cannot be used in this way because these abnormalities were only found in females. In the case of Huo et al. (2018), we can clearly see that there are sex differences in male and female rodents within the MAM model, specifically in the frontal cortical areas. In the current study, we found a reduction of 16% in the frontal cortical areas of the MAM-treated rats versus the saline-treated rats, as well as an overall reduction in the brain weights of MAM-treated rats when compared to the saline-treated rats. Our results are consistent with previous research findings that brain volume of various brain regions in male MAM animals are often reduced in comparison to placebo-treated groups (Hradtzky et al., 2012; Lodge & Grace, 2009). Other studies examining the impact in female mice have similarly found decreases in cortical volume, but no other studies other than the present study have found decreases in the volume of the frontal cortex in female

rats. Since rats and mice cannot be accurately compared in terms of brain abnormalities, the need for data in female rats is crucial to progress research in sex differences within the MAM model forward.

Furthermore, highlighting sex differences in both human patients and animal models is critical in understanding the disorder of schizophrenia. Although we were unable to find sex differences in this specific study because only female rats were analyzed, it is important to understand the stark differences between males and females in terms of the MAM model for schizophrenia. As previously mentioned with specific prefrontal and frontal lobe abnormalities, research suggests that female MAM-treated animals display decreased vulnerability of PFC function deficits when compared to males. Previous research suggests that MAM animals have consistently decreased thickness in the PFC and in total cortex size when compared to control animals (Chalkiadaki et al., 2019). This claim was supported by Chalkiadaki et al. (2019), whose research concluded that male rats were significantly more likely to present a manifestation of cognitive deficits in various tasks than female rats were after being exposed to MAM. However, it was shown that female rats were more likely to exhibit histological abnormalities that were not found in male MAM rodents, such as a decrease in ACC width and thinning in the IL (Chalkiadaki et al., 2019). The present study was not able to compare these findings between sexes but found a significant decrease in the size of the frontal cortical areas in MAM females compared to control females, in which MAM animals exhibited smaller frontal cortical area volumes. This evidence suggests that there are sex differences both in how dysfunctions are exhibited, as well as how each sex manifests their responses to schizophrenia.

Just as we observe sex differences between rats treated under the MAM model for schizophrenia, it is not surprising that these differences are also seen in human patients who have

been diagnosed with schizophrenia. Reductions in the grey matter of the human association cortex, particularly the PFC and ACC, have been identified as the most common histological deficits in schizophrenia patients (Chalkiadaki et al., 2019). In our analysis of the frontal cortical areas, the PFC and ACC areas were examined and were therefore concluded to play a role in the significant decrease in frontal cortical volume between our MAM and control rats. Our results emphasize and validate the consistencies in schizophrenia research in which abnormalities in these areas in both rat MAM models and human patients are seen.

Smith et al. (2013) uncovered a female "advantage" by finding that prenatal females scored significantly better than males on performance and full-scale IQ (minus verbal IQ). Performance IQ is designed to measure aspects of cognition such as logical thinking, motor coordination, and planning (Smith et al., 2013). A female "advantage" in these areas indicates significant sex differences in how brains develop under HI as well as the conditions that are likely to cause cognitive deficits. In order to find the best course of treatments for disorders such as schizophrenia, we must put more emphasis on the differences we see between males and females in how they respond to cognitive dysfunction and histological abnormalities in the brain.

Evidence suggests that females appear to have certain protections in place which make it less likely that they will exhibit some cognitive/behavioral deficits at the same rate that males do (Smith et al., 2013). One of these protections, as outlined by Hill & Fitch (2011), is neuroprotection. Neuroprotection refers to the ways in which the neuroanatomy of the female sex causes protection from cognitive damage and deficits (Hill & Fitch, 2011). In animal models, it has been shown that the presence of testosterone causes an exacerbation of cognitive response to HI, where estrogen has been found to have protective factors (Hill & Fitch, 2011). For example,

female animals have consistently displayed less tissue damage during stroke than male animals and females overall tend to have lower incidences of naturally occurring strokes (Hill & Fitch, 2011). This phenomenon is attributed partly to the protective effects of ovarian steroid hormone, which produces high levels of estrogen.

Sex Inclusion in Basic Research

Biological sex is one of the most important variables to consider when carrying out neuropsychological research. However, it tends to be the male sex is that is often prioritized in scientific research using animal models (Klein et al., 2015). There have been advancements to include the female sex equally in research. The National Institutes of Health (NIH), as of 2014, announced a policy that requires a balance of male and female cells from preclinical studies in future applications, attempting to make research more inclusive for the female sex (Klein et al., 2015). However, there are still barriers in place that keep the female sex sidelined in some research settings. Two common arguments made when excluding the female sex are that their hormonal cycles are too complex to control for and that the addition of female animals will skyrocket the cost of research (Sandberg et al., 2014). These misconceptions about the female sex create a prejudice against the use of female models, causing them to not be used as often in clinical research (Klein et al., 2015). Both of these misconceptions have been counteracted with sufficient evidence that disproves these arguments (Klein et al., 2015; Sandberg et al., 2014).

Sandberg et al. (2014) has argued that the deliberate exclusion of the female sex in clinical research is a direct Title IX violation, which states that no one can be excluded from programs that receive federal funding on the basis of sex. To combat the disparity of research pertaining to the female sex specifically in the MAM model, we chose to only analyze the tissue of female rats. Continuing to put emphasis on the specific abnormalities in the brains of female

rodents will hopefully aid in fighting the stigma against studying the female sex in schizophrenia research and in animal models.

Limitations

The present study was subject to several limitations. Firstly, some of the tissue samples we were working with were damaged and needed to be discarded during our study. Without a clear sample in which we could identify the frontal cortical areas, we were not able to properly measure them. Another limitation we faced was the size of the sample. We originally started with 19 rats, but due to the extent that some of the tissue was damaged or lost, as well as time constraints, we were only able to fully analyze a portion of these female rat tissue samples. Large effect sizes found within the data suggest that we still would have found differences with larger sample sizes. However, it is still possible that the small sample size could have skewed the results of the present study. We also faced limitations due to the COVID-19 pandemic, in which we did not have access to lab equipment to properly take new photos of tissue in a timely manner.

Further Research and Implications

The current study analyzed the brains of female rats and determined a 17% decrease in frontal cortical area volume in the MAM group when compared to the control group. Because of the limitations of the present study in terms of our sample size, future research should attempt to expand the sample and analyze the frontal cortical areas of the other female rats that we were not able to study in this data set. Further research should also measure frontal cortical areas of male rats using the point-counting method and compare results to the current study. This will help better understand sex differences in the MAM group's frontal cortical areas. Future studies should address the behavioral implications of these findings as laid out in Babcock et al. (2016),

in which behavioral consequences of MAM injection on E17 from the data set of rats analyzed form the sample size used in the present study. As previously highlighted, the role of estrogen in schizophrenia and its associated dysfunctions are instrumental in understanding sex differences in this disorder. The current study was not able to account for this factor in our histological analyses, so future studies including female samples should account for this variable from the beginning and analyze the role of estrogen in differences we see in frontal lobe areas.

Conclusion

The present study concluded that our sample of MAM-treated rats had significant body weight, significant brain weight, and significant frontal cortical area volume decreases. Brain abnormalities and their implications through the MAM developmental disruption model are well studied in male rats, but severely understudied in female rats. This is a problem because neuroanatomical differences between sexes in male and female humans with schizophrenia are observed, and if we want to better understand the implications of these differences, female rats need to be studied more extensively within the MAM model. This study is the first to analyze the frontal cortical areas of female rats specifically, and we hope that this research opens more doors to studying female rats within the MAM model. The MAM model can give us insights as to why these sex differences are present because if we are able to mimic these neuroanatomical changes through this model, we can begin to look at the impact of these observed brain abnormalities and draw comparisons to human patients. Through these comparisons between the MAM developmental disruption model and human schizophrenia patients, we are one step closer to developing more effective and specific treatments for schizophrenia.

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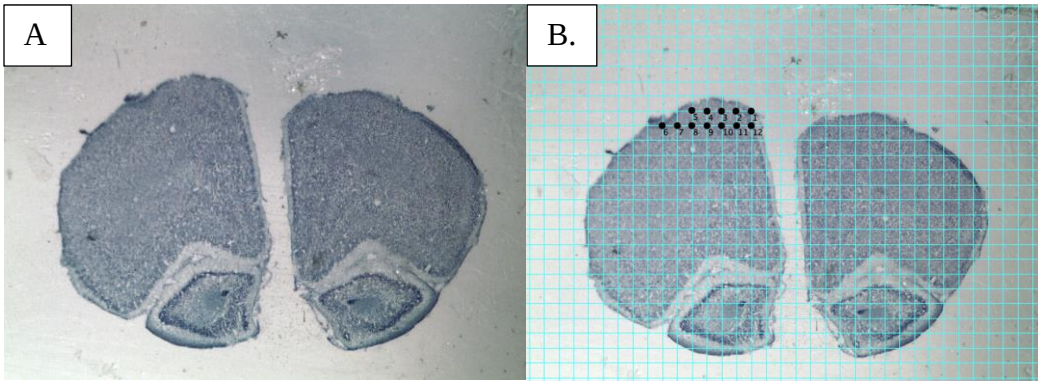
Figure 1

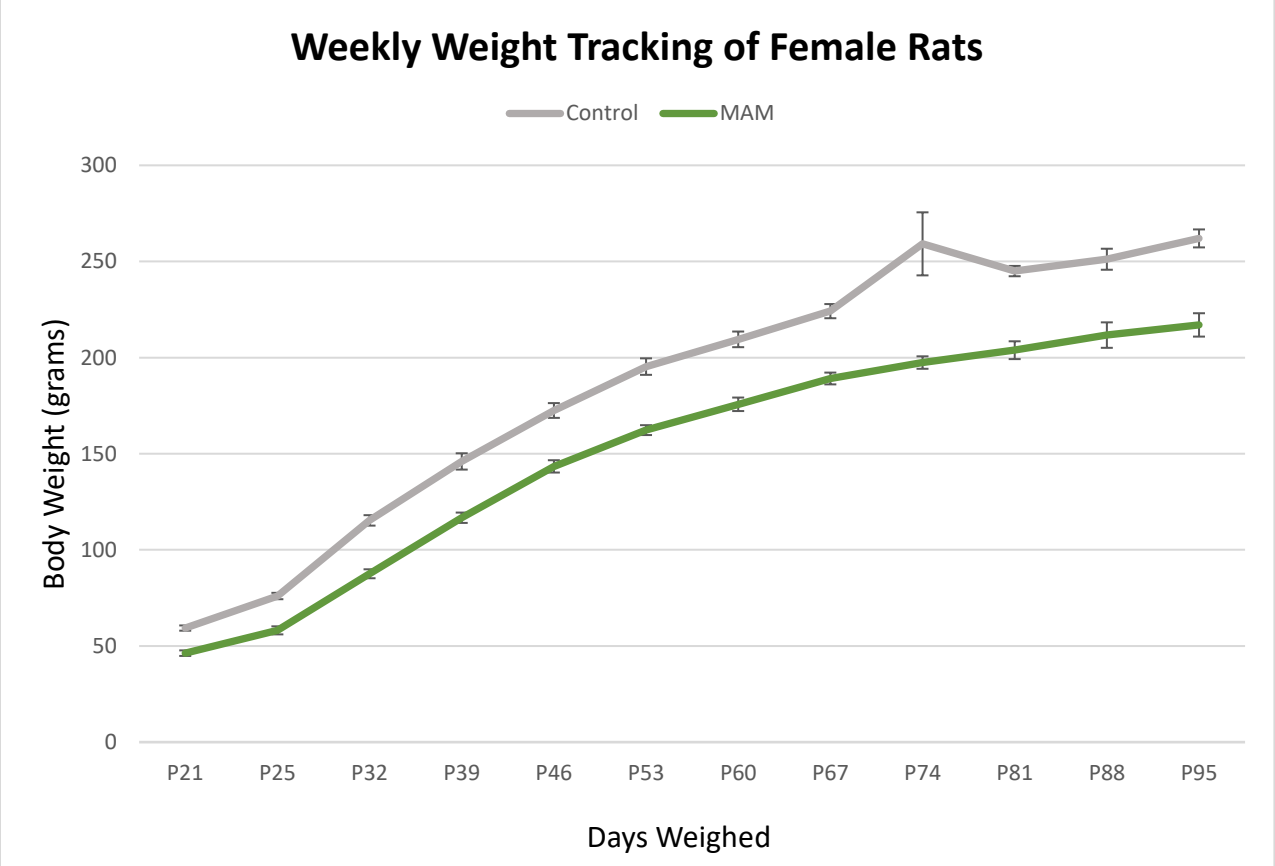
Rat Brain Histology with ImageJ Grid Overlay



Note. A. Thionin-stained section of the frontal cortical areas in a MAM rat. B. Grid overlay of the rat brain. Blue lines represent 178 pixels per millimeter with a 0.11 mm^2 area per point. Black dots represent sections counted. Stereological estimation and point counting were used to determine the volume of the frontal cortical areas on the rat brain tissue. Point counting is used to count the number of cross-section lines of the overlayed grid that fall within a brain structure that was chosen to analyze. These points were then converted to an area measurement using the known distance between cross-sections so that volume could be calculated. We analyzed tissue between bregma 6.12 mm - 2.52mm on a stereotaxic atlas.

Figure 2

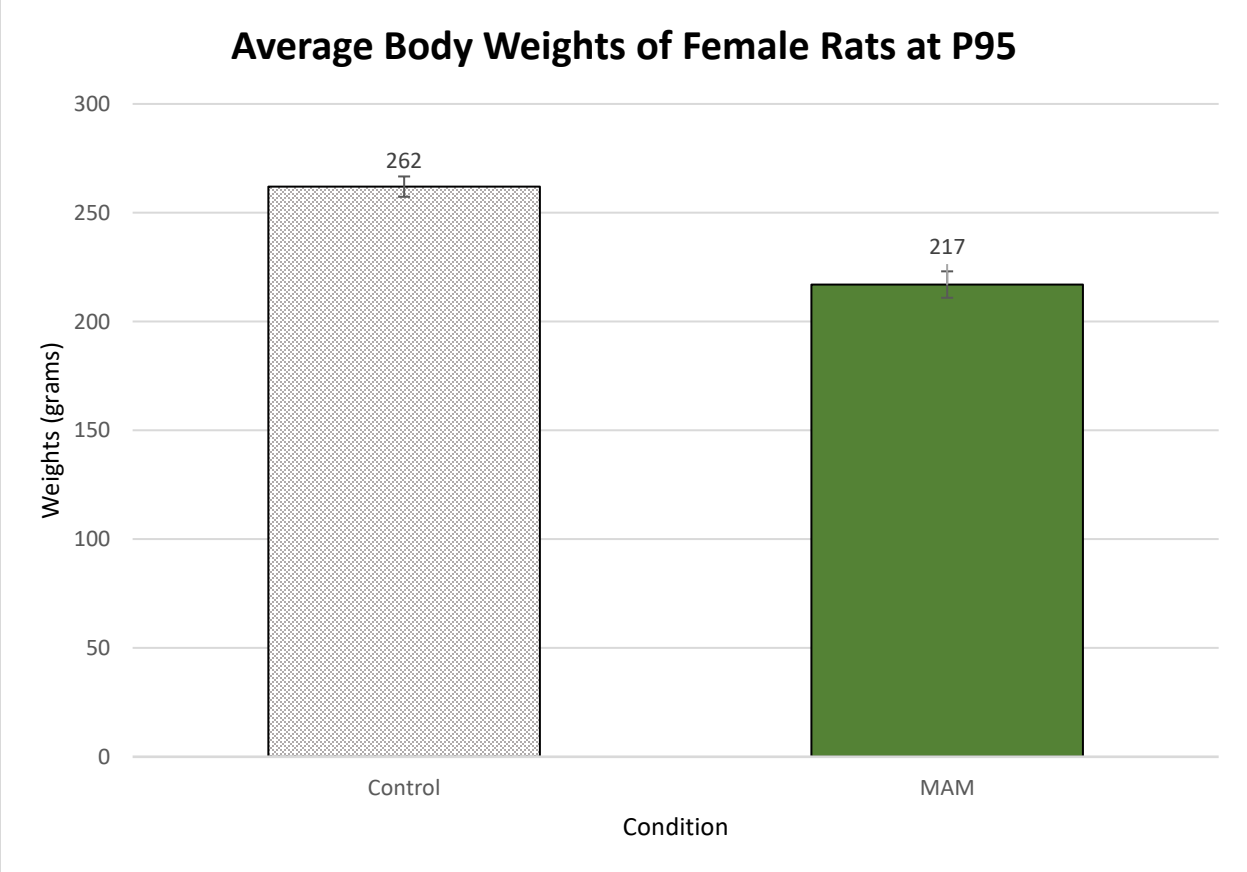
Weekly Weight Tracking of Female Rats



Note. The average body weights of rats were tracked between postnatal (P) day 21 – P95. The gray line represents control animals. The green line represents MAM animals. Error bars indicate the standard error of the mean. We found a main effect of day [$F(11, 121) = 403.9$, $p < .05$], suggesting that the rats grew steadily between P21 and P95. We found a main effect of conditions between-subjects [$F(11, 121) = 61.22$, $p < .05$], indicating that the two groups were significantly different from each other in terms of body weight. Lastly, we found a day-by-condition interaction [$F(11, 121) = 3.74$, $p < .05$], indicating that the rats were not increasing in weight in the same ways. All independent samples *t*-tests comparing days weighed to the MAM and control condition yielded significant results ($p < .05$).

Figure 3

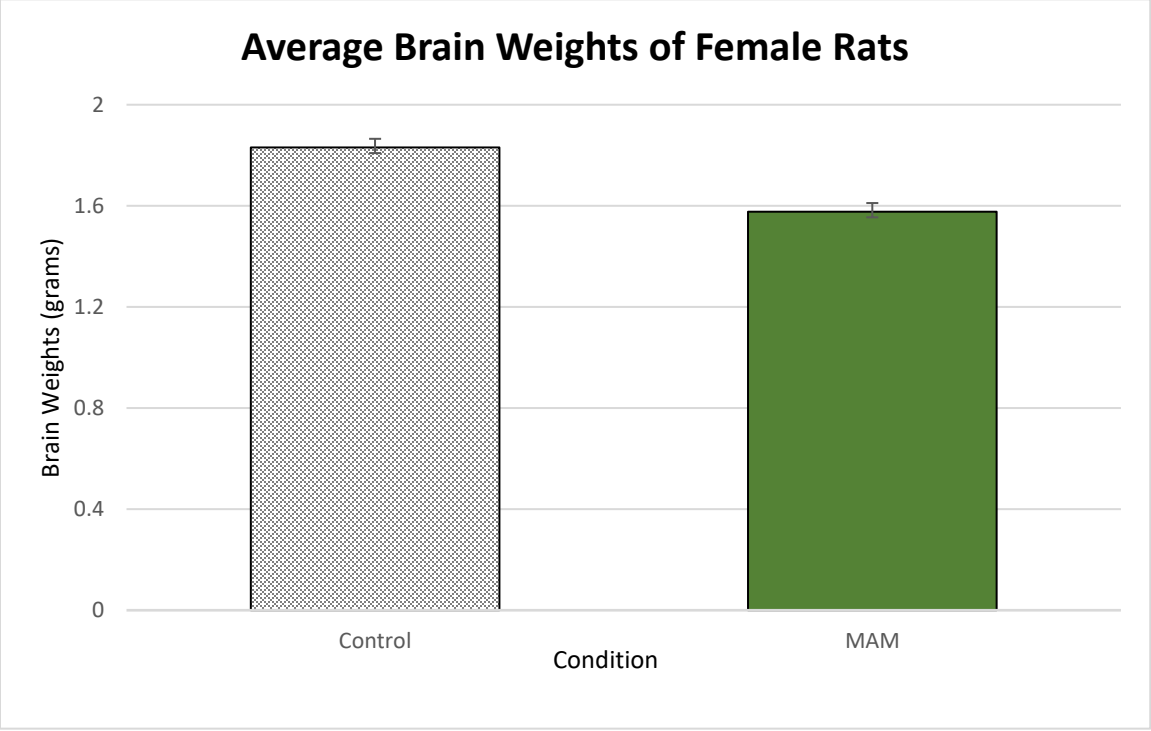
Average Body Weights of Female Rats at P95



Note. Average body weights on P95, the last day of weighing before sacrifice were measured to determine final group differences. The gray bar represents control animals. The green bar represents MAM animals. Error bars indicate the standard error of the mean. We found a significant decrease in body weights in the MAM group when compared to the control group, [$t(11) = 6.08$, $p < .05$]. A 17% decrease in the MAM group was observed when compared to the control group (control group: $M = 262$ grams, $SD = 11.44$; MAM group: $M = 217$, $SD = 16.07$), with a $d = 3.42$.

Figure 4

Average Brain Weights of Female Rats

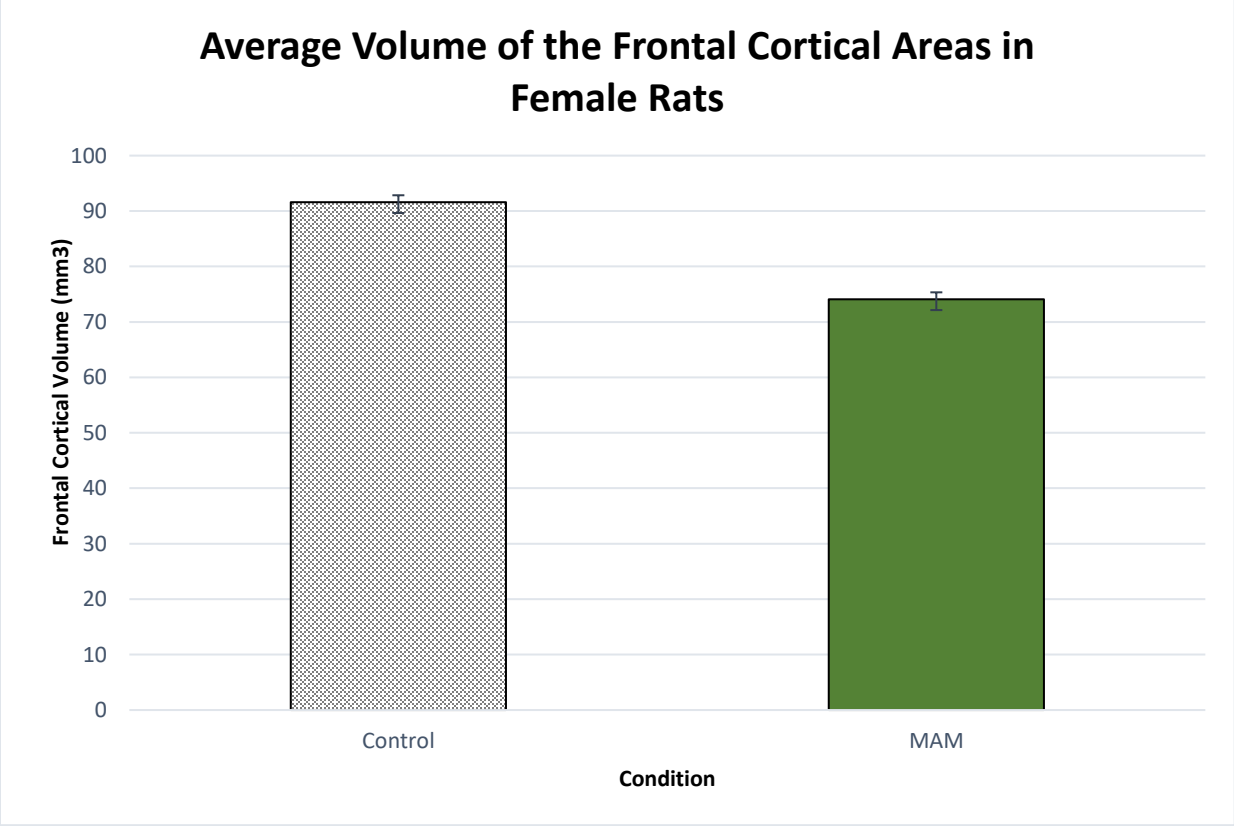


Note. The final brain weights of each rat were analyzed to determine the ending brain weight of each rat by condition. The gray bar represents control animals. The green bar represents MAM

animals. Error bars indicate the standard error of the mean. We found a significant difference between the MAM and control groups in terms of final brain weights, [$t(11) = 6.64$, $p < .05$]. These results indicate that the MAM and saline-treated groups showed a difference in brain weights based on their conditions. We observed a 14% decrease in the MAM treated group when compared to the control group (control: $M = 1.83$ grams, $SD = 0.084$, MAM: $M = 1.58$, $SD = .059$), with a $d = 3.54$.

Figure 5

Average Volume of the Frontal Cortical Areas in Female Rats



Note. The frontal cortical volumes of female rats were calculated using Cavalieri's estimator of volume after point counting analysis through ImageJ. The gray bar represents control animals. The green bar represents MAM animals. Error bars indicate the standard error of the mean. An independent samples *t*-test revealed a significant decrease in the frontal cortical areas of MAM-exposed female rats when compared to the control group [$t(11) = 6.08$, $p < .05$]. Specifically, a 17% decrease in frontal cortical volume was found in the MAM group compared to the control group (control: $M = 91.56$ mm, $SD = 3.07$, MAM: $M = 76.94$, $SD = 5.16$), with a $d = 3.38$

Appendix

This appendix incorporates the data and processes of the analyses completed in the present study. Our sample size consisted of 13 female rats, with six control rats and seven MAM rats. During researcher analysis of the frontal cortical areas in ImageJ, the conditions of each rat were not known. The conditions of each rat were only given after the analyses were complete to remove bias in the point-counting process.

The purpose of this appendix is to be both organized and transparent about the research practices carried out in the present study. Table 1 lays out the scale set up for each point-counted tissue in the ImageJ program. Table 2 lays out the body weights of each rat beginning at weaning (P21) and concluding at the point of sacrifice (P95). Table 3 describes the raw data from our body weight analysis, brain weight analysis, and final frontal cortical area volume of each rat. Brain and body weights were measured in grams, and the frontal cortical area volumes were

measured in millimeters cubed (mm^3). Table 4 lays out the means and standard deviations of each analysis by condition.

Table 1

ImageJ Scale Set-up

Distance in Pixels	178
Known Distance	1.0
Pixel Aspect Ratio	1.0
Unit of length	mm
Area Per Point	0.11 mm^2

Note. Table 1 shows an overview of how our scale for the ImageJ program. Each tissue sample used the same scale to keep results consistent.

Table 2

Body Weights of Sample Rats: Raw Data

Rat ID	15	16	17	18	19	25	26	27	28	29	36	45	66
Condition	0	0	0	0	0	2	2	2	2	2	2	0	2
P21	63	61	58	62	58	43	44	49	50	46	51	54	41
P25	82	77	72	79	72	37	57	66	66	62	66	72	53
P32	127	109	110	117	112	44	91	97	98	100	99	117	84
P39	162	136	137	142	144	74	123	123	127	132	127	155	111
P46	188	165	163	167	175	121	139	146	150	157	155	177	136
P53	208	182	186	191	201	146	160	155	172	169	171	204	163
P60	224	196	205	207	207	165	170	163	183	179	188	218	182
P67	221	210	225	226	225	181	178	183	197	193	198	238	194
P74	252	233	240	245	238	201	191	183	198	204	206	247	199
P81	252	236	240	249	242	210	194	185	205	205	221	251	207
P88	268	232	245	260	243	219	201	181	215	215	229	259	225
P95	266	246	269	272	249	217	204	192	225	225	234	270	229

Note. Code for conditions: “0” = Control, “2” = MAM. Days weighed (P21-P95) measured in grams.

Table 3

Female Rat Sample: Raw Data (N = 13)

Rat ID	Condition	Body Weight at P95 (grams)	Brain Weight at P95 (grams)	Calculated Frontal Cortical Volume (mm^3)
15	Control	266	1.94	93.36
16	Control	246	1.74	89.76
17	Control	269	1.85	96.25
18	Control	272	1.77	87.24
19	Control	249	1.78	91.15
25	MAM	217	1.57	77.03
26	MAM	204	1.55	75.71
27	MAM	192	1.60	86.79
28	MAM	218	1.68	79.62
29	MAM	225	1.47	75.57
36	MAM	234	1.61	71.47
45	Control	270	1.91	91.63
66	MAM	229	1.60	72.38

Note. This table lays out the raw data from each rat. Listed in this table are the Rat IDs, their condition (either control or MAM), body weights at P95 in grams, Brain weights at P95 in grams, and the total calculated frontal cortical area volume in mm^3.

Table 4.

Means and Standard Deviations of Each Analysis by Condition

Measure	Control	MAM
Mean Body Weights at P95 (grams)	262	217
SD Body Weights at P95	11.42	16.08
Mean Brain Weights at P95 (grams)	1.83	1.58
SD Brain Weights at P95	0.08	0.06
Mean Frontal Cortical Area Volume (mm^3)	91.60	74.09
SD Frontal Cortical Area Volume (mm^3)	1.25	1.95