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UNDERSTANDING SEX DIFFERENCES IN PATHOLOGICAL AGGRESSION WITH A MOUSE MODEL

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Introduction:

Antisocial behavior (ASB) is a commonly diagnosed personality disorder among young males as opposed to young females; the male to female ratio being 3:1 [1]. It is best described as chronic display of aggression that manifests during young adulthood. This pattern of aggression and hostility results in public violations, poor social encounters, and an overall dysfunctional society. The repercussions of this disorder seep into many facets of our community from a socioeconomical perspective, to mental health and criminal justice system burdens. ASB results in delinquency via aggression and hostility (including cases of weapon offenses as a means of perpetration along with homicidal offences) [2]. Regardless of how communally stirring this issue has become [3], the treatment options remain limited [4]. This issue is a wicked problem because it seeps into several aspects that make up a society. The unsatisfactory intervention methods highlight our lack of knowledge about the mechanism behind this behavior from a pathophysiological standpoint.

Background:

ASB has been seen more frequently in males and testosterone levels are closely linked with that find [5,6]. This study is focused on understanding the mechanism behind ASB through gene-sex interactions by comparing testosterone levels and genetic inclination towards low monoamine oxidase A expression (MAOA). Individuals who are genetically inclined to have low MAOA expression, and produce testosterone, have an increased risk for developing ASB [7] as seen below in ***Figure 1***. MAOA is the enzyme that activates the degeneration of dopamine norepinephrine, serotonin, and dopamine (DA) [8]. In tandem, low-activity MAOA expressers are genetically affected and influenced by testosterone (male vs. female) and this results in ASB [9].

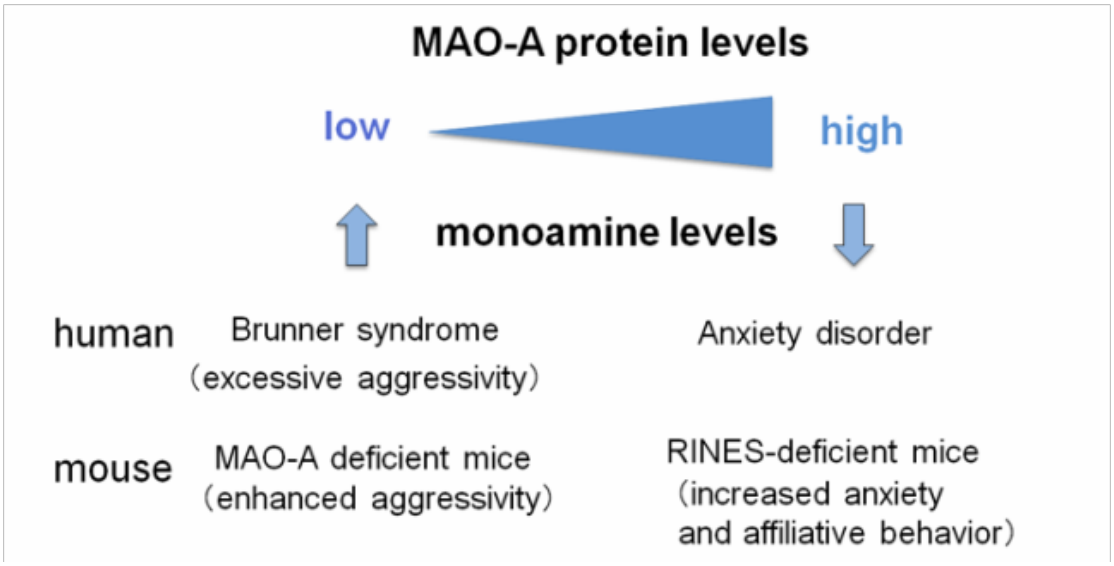

Figure 1. *MAOA. Human Gene Project. [accessed 2020 Mar 22].*

MAOA's role in aggression is mediated by DA efflux in the Nucleus accumbens (NAcc) from the preadolescent time frame onwards as demonstrated in new studies [10]. From the ventral tegmental area (VTA), DAergic mesolimbic projections are received by the NAcc and this modulates an incentivized response to chief environmental and social stimulants [11]. The rate-limiting enzyme for DA coalescence is tyrosine hydroxylase (TH) and testosterone enhances the synthesis of TH [12], as well as MAOA [13] during young adulthood [14].

With this background information in mind, we hypothesize that male, low-activity MAOA allelic variant's testosterone increases TH synthesis, in the mesolimbic neurons. Noting that this TH enhancement does not enhance MAOA, we can see that this disparity creates an imbalance among DA efflux to the NAcc while social stressors are present, leading to hostile behavior. In order to prove this mechanism true, we will use MAOA^{Neo} mice, a transgenic line developed in our laboratory that showcases low MAOA expression [15]. When compared to wild-type (WT) littermates, Male MAOA^{Neo} display greater aggression responses to social stress; indicating that they are keen candidates for this study being that they have low MAOA expression.

In general, MAOA deficiency and hypo-morphism in mice has been studied based on neuroimaging. Those experiments have laid serious groundwork for pre-clinical animal models that can later be applied to the humanistic mechanism. The first studies that contributed to neurobehavioral impact came from the MAOA knockout (KO) strain of mice. In this particular analysis, those mice were introduced to interferon beta cassette in exons 2 and 3 of the MAOA gene [15]. This caused a mutation that shifted the frame in which the DNA was originally read, causing the protein to be cut off prematurely. Consequentially, the phenotypic or behavioral qualities were extremely akin to those seen in those with Brunner syndrome which was first defined two full years prior to [16]. In that study, fully grown male mice who carried the mutation displayed intense aggression towards their male counterparts. This was also complemented with high levels of 5-HT and norepinephrine, which were evident in the first few weeks of life. The KO strain, as opposed to the normal WT strain, had levels elevated almost tenfold. These indications, along with the fact that the mutants exhibited a reduced reaction to typical stressors [17, 18], let people to associate these behavioral aberrances with those on the autism spectrum as well i.e. lessened tactile and acoustic sensation along with communication deficits etcetera [19].

On the same tandem with those discoveries, many children affected by Brunner Syndrome display abnormalities synonymous with autism. On this same thought, autistic patients who have MAOA-Low (MAOA-L) expression will have an increased severity for a number of symptoms, encompassing aggressiveness, communication deficiencies, and arousal regulation deficits. When considering the humanistic translation, many of experiments have exhibited that MAOA KO and $MAOA^{Neo}$ have Prefrontal Cortex (PFC) deficits [19]. Each of those mutated strains have been show to display disorganized cortexes and the significance of the PFC in aggression of MAOA KO is also denoted by proof showing that this behavioral abnormality is fixed by the genetic restoration of human MAOA in the forebrain region [20].

Methods:

The mice underwent several behavioral battery experiments such as social interaction, social recognition, dark-light box, elevated plus maze and ultrasonic visualizations. These tests are meant to highlight abnormal behavior among MAOA-L mice as opposed to normal mice via characteristic display. The characteristics these mice will show corresponding with each test above will include a self-isolation frequently, integrating oneself into a new group, higher risk-taking nature, and distressed calls. The set-up of these tests can be seen in the Appendix and the creation of the tools for these tests were majorly designed on Fusion and printed at the Marriott Library in the 3D-printing maker's space. The final test, ultrasonic visualization, was developed by a third-party company.

Figure 2. *Social Interaction & Social Recognition Arena*

Figure 3. *Dark Light Box Arena*

Figure 4. *Elevated Plus Maze Arena*

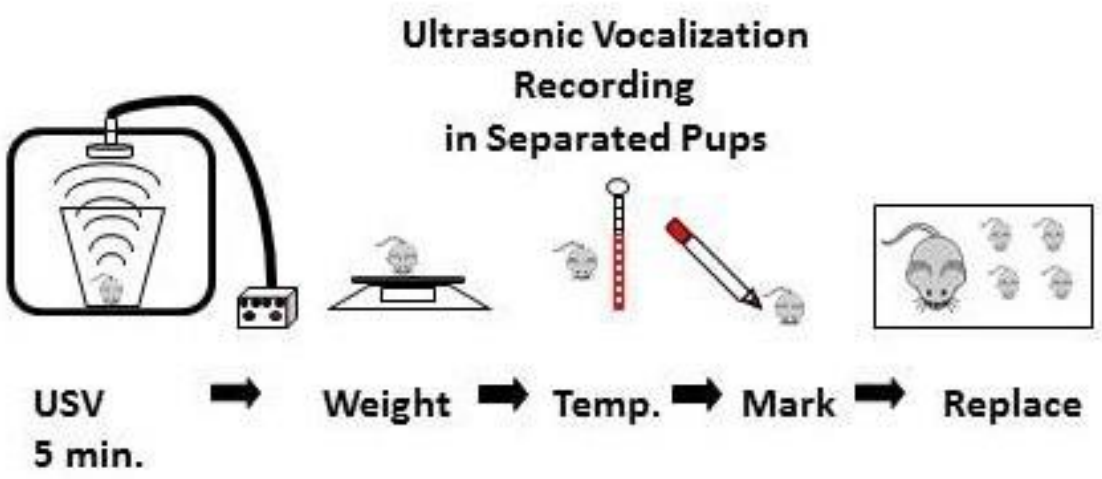

Figure 5. *Ultrasonic Vocalization Recording*

The reason individuals express ASB has to do with a combination of genetic variables i.e. MAOA levels and/or reinstatement of those said levels along with environmental factors. The MAOA levels have been detailed, but how do we qualify environmental factors to develop this kind of testing? Early life maltreatment was first used to create this environmentally factored, aggressive phenotype. This model ranges from maternal separation at a young age, all the way to daily intraperitoneal injections. These are meant to induce the same effects of neglect and physical abuse seen in children. These events usually take place in pups in the first three weeks of their life on a partially random timetable to imitate real life abuse. These environmental factors illicit aggressive responses developed in adolescence, that align with human findings [21].

In order to determine by what method MAOA genotype alters sex differences in DA yields, we must first look at what has been previously used in this particular area of study. Brain-regional expression of TH and MAOA will be quantified by a western blot and mapped by immunohistochemistry post male and female $MAOA^{Neo}$ sacrifice. It is expected that male $MAOA^{Neo}$ mice will showcase higher TH, but not MAOA in DAergic cells [8]. Also, it is imperative that we are able to understand how to categorize the manner in which the exchange between MAOA and sex differences alters DA release in the NAcc during violent and/or abusive

situations takes place. In previous studies and research regarding the topic, male (gonadectomized and sham-operated) and female MAOA^{Neo} and WT mice were placed in the presence of unfamiliar mice, and their changes in hostile behaviors were recorded, measured, and correlated with DA levels in the NAcc (as measured by micro-dialysis) [12]. This stressor will increase the levels of hostile and aggressive behavior, as well as mesolimbic DA release, in sham-operated MAOA^{Neo} males, but not females or castrated males.

MAOA^{Neo} mice are available in our colony and will continue to be bred and genotyped [22]. Given that MAOA is on the X chromosome, there are 2 genotypes in males (WT and MAOA^{Neo}) and 3 genotypes in females (WT, heterozygous, and homozygous MAOA^{Neo} mice). The studies in Aim 1 will require [10 animals x 2 time points/group x 5 genotypes] = 100 animals. To adequately power the studies in Aim 2, we will use [20 mice/group x 2 sexes x 3 groups (sham males, castrated males and females)] =120 animals. We will not include gonadectomized and heterozygous MAOA^{Neo} mice in these studies to focus on the key questions and maintain statistical power; however, should our results point to significant differences between homozygous MAOA^{Neo} and WT females, we are ready to include heterozygous and ovariectomized mice. To control for litter effects, no more than 1 mouse per sex and genotype will be assigned to the same group. All proposed techniques will be performed as described in our previous studies [monitoring of hostility and aggression [23], castration [24], western blotting [25], immune-histochemistry (26), as well as micro-dialysis and HPLC analyses of DA [24, 27]. To ensure rigor and reproducibility, all studies will be performed by personnel blinded to the experimental groups.

Barriers:

All chemicals, animals and equipment necessary for the completion of these studies are readily available in our laboratory. We do not anticipate any struggles that may obstruct

experimental achievability given our experience with these procedures. A potential problem may be that, during the studies in Aim 2, mice may lose the micro-dialysis probe during hostile encounters due to brawling. In this unlikely event, we will exchange those animals with other conspecifics, so as to reach the necessary numbers in each group.

Results:

Due to the repercussions from COVID-19, our research was put on hold and this hindered any further data collection. This cannot be remedied until the pandemic is over. We do not have enough data to draw conclusions. However, this time can be used to make figures from the data collected thus far and draft up potential take off points for the future.

Discussion:

ASB is a clinical diagnosis branded by aggression, violence, drug use, thrill-seeking behavior, along with delinquent behavior. While childhood and adolescent maltreatment have widely been approved as factors that contribute to this condition, there have been major studies contributing to a communal agreement that MAOA is a major contributor as well. Those with low levels of MAOA have flagged to be at an elevated risk for ASB development. Particularly in men with a background of abuse and neglect, MAOA-L variants have been closely correlated. The growing succession of research prompts us to think that the MAOA polymorphisms have to do with the functional and structural brain alterations in ASB. Future genetic imaging will aid us in better understanding the mechanism behind this structure and functionality. The preclinical and clinical models have indicated that low MAOA expression and early life stress create ASB vulnerability and have also highlighted the vital role that 5-HT2A receptors play in the process. This may even suggest that HT2RA polymorphisms may be responsible for how ASB is moderated.

Future Directions and Implications:

Being that the project goals were put on hold, much work still needs to be completed in order to successfully analyze the data collected thus far. The goal will be to continue further research and either falsify or prove my hypothesis. As of now, the only approach we would maybe differ in would be the white noise in some of the experiments. A white noise machine would help alleviate any external communication and/or signaling to the huddle and would allow for clearer test results. If our hypothesis is proven true, that sex differences do have an effect on pathological aggression, specifically that MAOA-L males will have a TH increase in the mesolimbic areas leading to overall hostility. If this is the case, this will further our understanding of the mechanism behind antisocial behavior disorder and will inherently allow for drug treatment trials to help remedy this issue.

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APPENDIX

Arena	Purpose
MAOA Diagram—Figure 1	*This graphic displays low MAOA amounts in a human equate to Brunner syndrome, whereas low amounts in a mouse lead to acute aggression. It also displays that high amounts of MAOA in a human equate to anxiety and in a mouse, contributes to RINES-deficient mice.*
Social Interaction—Figure 2	*Social interaction is setup by placing one pup group at an end of the arena. One mouse is picked from the litter and is placed at the other end and the latency is measured between when it is placed and when it reaches the huddle again. This shows a willingness to be integrated into a group as opposed to self-isolation. A normal mouse would reintegrate itself into the group.*
Social Recognition—Figure 2	*Social recognitions setup by placing two pup groups at opposite ends of the arena. One pup from one little is placed in the center and the latency is measured between when it is placed and when it reaches the huddle along with whether or not that huddle is of its own origin. This shows the risk-taking between an unfamiliar huddle versus a known group. A*

	normal mouse would veer towards the known group.
Dark Light Box— Figure 3	*The Dark light box is set up by placing one pup from the huddle X distance away from the dark entrance and the latency is measured between the drop-off time and the entrance time. This shows the risk-taking nature of the animal to be without cover and in a bright space as opposed to a covered dark area which is what normal mice prefer.*
Elevated Plus Maze—Figure 4	*The elevated plus maze is setup by placing a mouse in the center and measuring their time in the open fields, middle field, and closed fields. This shows how daring the animal is to step outside of the safe and enclosed spaces whereas a normal animal would spend a majority of the time in the closed spaces.*
Ultrasonic Vocalization—Figure 5	*Ultrasonic vocalization is set up by separating the pup from its huddle and measuring the vocalizations it makes while separated. A normal mouse will have certain sounds and pitches.*