BIOL 463 – Final Project
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Maternal genotypic effect on offspring activity level of 3xTg-AD mice

Introduction:

Alzheimer's disease (AD) is the most common forms of dementia which is characterized as the detrition of cognitive abilities such as memory, thinking and motor capabilities (Blaney *et al.* 2013). It is known as a frontotemporal disorder which occurs due to damage to neurons in parts of the brain called frontal and temporal lobes (NIH 2015). As the neurons in the frontotemporal region of the brain die, this area of the brain shrinks by atrophy. This atrophy results in symptoms such as unusual behaviour, emotional problems and difficulty with locomotion or motor control (NIH 2015). Specifically, circadian disturbances of fragmented sleep-wake pattern and sun-downing (changes in behaviour depending on the time of day) are commonly associated symptoms with AD (Bliwise 1993; Sterniczuk *et al.* 2010).

Triple transgenic mice (3xTg-AD) are a model for AD, mimicking the human AD, as these mice exhibit age related cognitive decline, amyloid beta plaques, neurofibrillary tangles and neural cell death (Blaney *et al.* 2013). These characteristics are due to the accumulation of amyloid-β protein (Aβ) and hyperphosphorylation of tau protein (Hardy& Selkoe 2002; Kowalska 2004). 3xTg-AD mice are currently the only model systems that exhibit both Aβ and tau pathology, and mimic human AD (Sterniczuk *et al.* 2010). However, before these distinct traits associated with AD, more general behavioral and cognitive decline is seen which progresses with the development of the disease such as a fragmented sleep–wake pattern.

In humans, behavioral and physiological factors, like the sleep-wake pattern (or circadian pattern), show cycles that correspond with a 24-hour clock (Hastings *et al.* 2008). Mice are nocturnal animals where they have peak activity within the first hour of the dark phase. As a result, their motor activity changes in correlation with the circadian cycle (Dunnett *et al.* 2009). Circadian pattern disturbances, like sleep disorders, are associated with aging and are specifically seen as early identifiers of AD (Wu and Swaab 2007). This disturbances or changes to the sleep-wake pattern is due to disruption of the primary mammalian circadian clock, a decrease in input to the suprachiasmatic nucleus. This is seen when there is atrophy in the suprachiasmatic nucleus and a decrease in the number of vasopression and vasoactive intestinal polypeptide containing cells in the suprachiasmatic nucleus (Wu and Swaab 2007). Individuals with AD show a degeneration in the ability to send neural information to the the suprachiasmatic nucleus and as such results in atrophy seen in this region. As such, disturbances of the circadian cycle in mammals is associated with developing AD (Sterniczuk *et al.* 2010). Many attempts have been made to create a mouse model that would show similar neuropathology and behavior as seen in human with AD. Of these models, triple-transgenic model 3xTg line has been the most comparable to humans (Sterniczuk *et al.* 2010).

3xTg-AD mice have been shown to display changes in circadian patterns even preceding other known behavioural symptoms associated with AD. The disturbance in the circadian rhythm of 3xTg-AD mice was determined by monitoring the difference in locomotive activity of the mice as compared to wild-type mice. In the of 3xTg-AD mice there were significant changes in the pattern of locomotive activity with increase amount of time spent active during typically inactive phases of the 24-hour cycle. (Sterniczuk *et al.* 2010).

It has been shown that by a cross-fostering paradigm maternal genotype influences the behavioral development of 3xTg-AD mice. It was found that postnatal maternal effects of rearing resulted in changed phenotypes of the 3xTg-AD mice. The pups that were reared by wild-type mothers at the end of the testing period, weighed more and had more horizontal activity than those reared by transgenic mothers (Blaney *et al.* 2013). This difference in post-natal care results in a change in the phenotype of the pup. Developmental programming or the early environmental influences that a pup is subjected to can have a profound impact on later development, structure and function This study will look into the developmental programming effect that maternal genotype plays in the prenatal stages of development, which has not been studied in relation to the circadian pattern 3xTg-AD mice. This study works off the precedence that maternal genotype having been shown to influence offspring phenotype in both pre- and postnatal cases in mice (Heijtz *et al.* 2011; Blaney *et al.* 2013).

The purpose of this study is to determine the role of the maternal genetics in the onset of AD related behaviors in the 3xTg-AD mice, specifically through looking at changes in circadian patterns by monitoring locomotive activity. Specifically, this study looks to answer if the maternal genotype is sufficient to change the 3xTg-AD mice activity level phenotype in relation to the mammalian circadian cycle. It is predicted that maternal genotype plays a role in development that can affect or change outcomes of locomotion behaviors associated with the AD mice model, 3xTg-AD.

If it can be shown that circadian patterns as monitored by locomotion phenotype in 3xTg-AD mice can be affected by maternal genotype in the prenatal environment of the womb, this could change the way we look at AD. This could act as a stepping stone to open doors for research avenues that are currently unexplored by showing that prenatal environmental plays a role in AD phenotypes. It should be noted that using transgenic mouse models do have their limitations as AD is a syndrome rather than a disease of specific mutations so though there are correlations in the types of phenotypes and genetic changes in this mouse model and humans, the model is not without flaw (Bryan *et al.* 2009). By first looking at an easily characterized phenotype, activity level, other phenotypes related to the symptoms as well as the developmental pathway of AD can be looked at in relation to prenatal developmental programming and the effect of maternal genotype. Perhaps this will lead to the characterization of the maternal factors that influence AD *utero*. These results could have major implications for the understanding of AD in humans and possibly allow for the exploration of *in utero* prevention for mother's whose genotypes show high risk of inducing AD related symptoms.

Experimental Design

In previous studies on circadian changes in 3xTg-AD mice, male and female 3xTg-AD mice have shown alterations of their circadian pattern however males show a higher level of activity in the day while females have a decrease in activity during normally active phase (Sterniczuk *et al.* 2010). Due to this variance among the sexes, data will be collected such that male and female will be kept as separate categories. In future studies, male and females may be compared in order to reveal possible sex specific phenotypic changes to the activity levels of 3xTg-AD mice. In both the control and experimental groups, the number of lines of inbred mice used will be those that give statistically significant results. As male and female will be separately considered, multiple IVFs will be set up, ultimately a random sample of 10 male and 10 female for each control and experiment will be used to collect information (such that there is a total of 40 mice across all groups). There will be 4 groups in the experiment (experimental testing will commence at age 6 months): control wild-type female, control wild-type male, experimental 3xTg-AD female and experimental 3xTg-AD male. It should be noted here at that wild-type refers to non-transgenic mice that do not have any neurological or behavioral abnormalities.

The specific null and alternate hypotheses from this experiment are:

H_o: The mean running activity from subjective day and the subjective night is the same for all groups. (ie. variance is equal to zero)
H_A: The mean running activity from subjective day and the subjective night is different for at least one group from the others. (ie. variance is not equal to zero)

Control Group:

Take 20 inbred strain female 3xTg-AD mice (that can be purchased from the Jackson Laboratories (Bar Habor, ME, USA). Oocytes that are unfertilized will be removed from the mother of the same line. Next *in vitro* fertilization of the oocytes with sperm from the same father will be used in order to make fertilized embryos that are genetically similar. In this control group, re-implant the IVF back into the mothers.

Experimental group:

For the experimental group, 20 inbred strain female 3xTg-AD mice, again from the Jackson Laboratories, will be used and unfertilized oocytes will be removed from this same the mother. IVF with sperm of the same father to create genetically similar fertilized embryos. Now instead of re-implanting the IVF into the mothers these fertilized embryos from the 3xTg-AD mice will be transplanted into inbred lines that are wild type mice.

Both the control group and the experimental group will be birthed normally, but as it is known that post-natal care has an effect on the behavior of the 3xTg-AD mice, all pups will be fostered by the wild type female mice (Blaney *et al.* 2013). Mothers will be removed from cages as all of the pups are transferred the to cage of the 20 3xTg-AD female mothers, taking care to label the

pups that were implanted from wild-type mice mothers with an FDA-approved pigment on the tail so that they may be identified during observation (Careau *et al.* 2012; Dunnett *et al.* 2009). Eliminating the variable of rearing leaves maternal genotype *in utero* as the main different between the control and the experimental groups – experimental group is fostered from wild-type mothers *in utero* is the main variable left.

Average running activity will be calculated for the subjective day (circadian time; CT 0–12) and subjective night (CT 12–24). Locomotion activity will be determined by wheel-running activity level and be monitored by a computer running the Clocklab data collection data software package and activity charts will be created with the Clocklab Analysis software, modelled after previous circadian change studies in 3xTg-AD mice (Sterniczuk *et al.* 2010). Using wheel-running as the gage of activity level is advantageous because it gives high-quality data that is able to be full automated. Mice also do not need to be moved to a new location that would possible lead to increased anxiety that would affect the data (Dunnett *et al.* 2009). Additionally, it has been shown that wheel running can actually decrease stress compared to other forms of testing such as mazes that increase anxiety/stress levels (Selam *et al.* 2009). Mice will be kept in for light-dark cage condition for 10 days, length of testing period at 6 months of age. Days will be based on the circadian time: day CT0hr-12hr and night CT12hr-24hr.

Possible Outcomes:

It is predicted that results would show support for the H_A hypothesis. To appropriately test this specific hypothesis, an ANOVA statistical analysis test will be employed using the Clocklab Analysis software generated data to determine if there are significant differences between the four groups.

If the variance between groups is equal to 0 then the observed variance between groups that is more than expected by chance as predicted by the genotypes of the surrogate mothers. If the variance between groups is not greater than what is expected by chance, then the groups are not statistically different from each other, rejecting the H_A hypothesis. This would mean that the foster mother's genotype is not sufficient to change the pup's circadian cycle related locomotive activity. If the variance between the groups is greater than chance as predicted by genotypes, then one of the groups is statistically different from at least one of the other groups. This would be mean that foster mother's genotype is sufficient to chance the pup's circadian cycle related locomotive activity. The important conclusion from this result would be that pups born from genetically different foster mothers have a statistically different circadian related locomotive activity level than different groups. It can be inferred that there is a genotypic factor that is acquired during embryogenesis or development from genetically dissimilar foster mothers that leads to the pups she carries *in utero* to display a phenotype that is either more or less disruption of circadian cycle as measured by sleep-wake locomotive activity.

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