Question 1

Alzheimer disease is a devastating and widespread issue (Mathers, Boerma, and Ma Fat 2013), the etiology of which is just now being understood. Current research has supported the idea of a seeding nucleation mechanism by pathogenic species of beta-amyloid (AB) to induce amyloid dysfunction in the brain leading to Alzheimer disease (Hamaguchi et al. 2011; Fritschi et al. 2013; Walker LC et al. 2013). This hypothesis has been supported by studies indicating the ability to induce Alzheimer's-like symptoms simply by injecting homogenate containing pathogenic AB from an Alzheimer patient into susceptible mice (Morales et al. 2012; Guo and Lee 2014). Currently, no one has tested this in vitro to confirm if cell-to-cell transmission of AB pathology is due to neuronal involvement.

Recent work has also outlined robust sex differences in the progression of Alzheimer's disease. Women display higher prevalence (Li and Singh 2014), faster progression (Carroll et al. 2010; Lin and Doraiswamy 2015), more global pathology (Barnes et al. 2003; Barnes LL et al. 2005) and greater cognitive decline, even in the presence of treatment (Li and Singh 2014; Rahe et al. 2015). However, men do display higher mortality rates (Gambassi et al. 1999) as well as a higher prevalence of mild cognitive impairment (Mielke, Vemuri, and Rocca 2014). These sex biases may be the result of estrogen and progesterone loss during menopause in females or loss of androgens in males (Li, Cui, and Shen 2014; Sherwin 2012) but, early life environment should not be ruled out as a factor (Penaloza et al. 2009). These sex biases likely involve the highly differentiated endocrine history of males and females (Vandenbergh 2003) along with their genetic make-up (Penaloza et al. 2009). Studying the multiple effects of sex on disease progression is a necessary step in understanding and treating Alzheimer's in all individuals.

Here, I hope to examine sex differences in neuronal AB transmission and cognitive impairment in a mouse model of Alzheimer disease in vitro and in vivo. When expressing the human wild-type amyloid precursor protein (HuAPPwt) in mice, progression to Alzheimer-like pathophysiology may be induced at any age by introducing pathogenic AB from an Alzheimer patient (Morales et al. 2012). By culturing tissue from HuAPPwt embryos and producing three interconnected populations of neurons I plan to examine their ability to transmit pathogenic AB through cell to cell transfer. By collecting tissue prior to hormonal intervention, I will model the effects genetic sex may have on Alzheimer pathology. Then, the first surge of sex hormones that lead to a prenatal organizational period will be examined and their effect on AB transmission determined (Penaloza et al. 2009). Further tests will see whether masculinization of female tissue can provide equal protection as it does for male tissues in vivo by examining cognitive impairment and spread of AB pathology. **<u>I hypothesize that prenatal genetic and hormonal masculinization of neural tissue decreases rate of cell-to-cell transmission of pathogenic AB proteins</u>.** I predict that masculinization of XX cells through prenatal treatments of androgen will rescue the susceptible female phenotype. **I therefore expect restricted spread of pathology and less cognitive decline in masculinized animals and tissues through-out this study.**

<u>Significance:</u>

Late onset Alzheimer's disease is the most common age-related cognitive impairment reported in individuals older than 65 and with a large number of individuals about to enter this age group (Mathers, Boerma, and Ma Fat 2013) it has never been more vital to understand the disease. Recent examinations that include sex as a factor have found several female-biases worth studying (Mielke, Vemuri, and Rocca 2014; Li and Singh 2014; Lin and Doraiswamy 2015). Already, new advances in our understanding of Alzheimer's have developed novel questions to be applied to sex difference Alzheimer research.

Question 1

We now know Alzheimer's disease is partly characterized by the abnormal accumulation and spread of insoluble AB proteins. Normally these proteins play a role in microtubule stability and are cleared away by several proteins which degrade or sequester the molecule (Fritschi et al. 2013). Pathogenic AB are resistant to being cleared and it is thought that presence of a pathogenic AB can lead to the pathogenesis of other AB molecules. This then leads to the spread of pathophysiology through-out brain regions (Fritschi et al. 2013; Guo and Lee 2014). Interestingly, mice expressing the wild-type human amyloid precursor protein do not display Alzheimer-like spread of pathology without perturbation. Injection of an Alzheimer disease patients' AB protein homogenate is necessary to induce pathology, indicating the protein can act similar to prion proteins when injected directly into the brain (Morales et al. 2012). So, cell to cell transfer of a pathogenic protein is likely a cause of Alzheimer disease. This mode of pathogenesis may have a sex difference given the robust sex differences in disease presentation (Li and Singh 2014). Understanding these sex differences is critical for treatment and diagnosis.

Interestingly, the burden of Alzheimer disease is greater in the female population (Li and Singh 2014). Previously, the phenomenon of female-biased cases of Alzheimer's was attributed to the fact that women often live longer in our society but recent work demonstrates that this cannot be attributed to their increased lifespan (Dye et al. 2012). Although, their longer life expectancy means they will likely be affected by the disease for a longer period of time than male counterparts. Unfortunately, not only are rates of disease progression faster in women (Rahe et al. 2015; Li and Singh 2014), women have more cognitive impairment than men with equal amounts of pathophysiology (Mielke, Vemuri, and Rocca 2014; Barnes LL et al. 2005). Still, men do have higher mortality rates compared with females and more males present mild cognitive impairment (Gambassi et al. 1999; Mielke, Vemuri, and Rocca 2014). These data impart the need for sex-difference research in the cell-to-cell transfer of pathogenic AB species.

Innovation:

Sex differences are an important part of neuroscience research (Cahill 2006). Already described are huge differences in the presentation of Alzheimer disease between males and females and therefore in order to develop effective treatments and diagnostics for both men and women, an understanding of the sex-typical pathogenic process must be gained. Mouse models have also shown the same female-biased burden of disease (Carroll et al. 2010) as seen in humans (Li and Singh 2014). This indicates that mice may be implemented as models of human sex differences in Alzheimer disease. Research attempting to understand sex differences in diseases such as Alzheimer's has recently been solicited by the NIH (Clayton and Collins 2014) and this is necessary to assist all those affected by this disease. Here I will begin the examination of sex differences by looking at two early-stage developmental time points in an effort to provide critical information of disease progression and sex-biased vulnerabilities.

To do this, I will develop a new model for testing sex differences in AB transmission in vitro through adapting studies for transmitting pathogenic α-Synuclein in vivo and in vitro (Luk et al. 2012; Tran et al. 2014). Using wild-type amyloid precursor protein expressing mice to form cell cultures which are then inoculated with homogenate containing AB from an Alzheimer patient or by injecting the AB homogenate directly into the brain (Mucke et al. 2000), I will produce disease presenting tissue. By comparing sexes I will be developing a basis for sex difference research in a novel Alzheimer disease model along with supporting the prion nature of pathogenic AB.

Although other models exist, I feel using mice expressing the human wild type AB protein will be the most efficacious model for this study. Using human wild type expressing AB

proteins provides insight to non-familial late presenting Alzheimer disease, the most common form. This model will also take advantage of the recent understanding that AB proteins have prion-like abilities (Fritschi et al. 2013) to propagate pathology in otherwise healthy mouse tissue (Morales et al. 2012). This is well supported in mouse models in vivo (Guo and Lee 2014) but has not been shown to require movement by neuronal cells. However, given that disease pathology in the brain moves along interconnected regions (Morales et al. 2012) it seems likely neuronal transport of pathogenic AB occurs. The regional spread of pathology is defined well for Alzheimer disease (Barnes LL et al. 2005), indicating there may be specific differences in regional resistance to AB pathology (Bero et al. 2011). How these interact with sex differences has never been examined. Here I plan the first examinations into how sex differences may alter specific brain regions for later interventions in AB dysfunction.

Using mice provides inroads to many other transgenic manipulations not available to rat models of Alzheimer disease for studying sex effects (Eckman et al. 2003; Carroll et al. 2010; Breedlove 1992). Studying this effect in other mutant strains may indicate various characteristics of AB's prion like tendencies, for example, in 3xTg-AD mice AB accumulation differences has already been tied to neonatal sex steroid levels. This model mimics the familial-early-onset form of Alzheimer disease. Studying this transgenic animal using the in vitro model presented here would provide knowledge on how seeding AB dysfunction can increase the rate of pathological AB accumulation in people with this type of Alzheimer disease. The use of transgenic animals will also allow the study of other aspects of AB pathology. The abnormal clearance by certain enzymes may be examined through genetic knock-out. You could use knock-outs of clearance factors such as neprilysin (Iwata et al. 2001) or insulin degrading enzyme (Farris et al. 2003). These would allow an understanding of the mechanisms by which sex is able to alter resistance to disease.

Approach:

In human studies (Li and Singh 2014; Barnes LL et al. 2005; Gambassi et al. 1999) and mouse studies (Carroll et al. 2010), sex differences are exhibited in the spread and toxicity of AB pathology. Here I hope to examine one aspect of abnormal aging in the brain, the transmission of pathogenic AB proteins from neuron to neuron. How genetic sex and the prenatal hormonal environment alter this transmission will be the focus of this study.

Aim 1: Determine sex- and regional-specific transmission rates of AB pathology before and after the first sex-hormone organizational period.

Animals: All animal protocols will follow approved IACUC guidelines. Female HuAPPwt mice will be placed with male HuAPPwt mice (purchased from Jax; strain: 004662; Strain name: B6.Cg-Tg(PDGFP-APP)5Lms/J) overnight and presence of a vaginal plug will indicate the first day of gestation. At gestational day (GD) 10 and 17, pregnant females will be killed by CO_2 asphyxiation followed by cervical dislocation. Gestation day 10 mouse embryos will have only been exposed to one aspect of sex determination, genetics (Du et al. 2004), while in order to examine the effects of the first male testosterone surges, the brains of embryos at GD 17 (Vandenbergh 2003) will be implemented. Sexing of embryos will be done using Zfy and Zfx primers (for details see: Penaloza et al. 2009), two bands (124-bp for Zfy and 134-bp for Zfx) indicating the presence of a Y chromosome.

Human AB homogenate: A sample from the cortex of a clinically diagnosed Alzheimer patient will be removed 2 hours postmortem. Histological analysis will be performed (see Beta-amyloid imaging below: Klunk et al. 2002) after collection to confirm diagnosis. Samples will be homogenized while in phosphate buffered saline mixed with a protease inhibitor (Morales et al.

2012). A manual sonicator (Sonics-Vibra) will be applied for 3 seconds at 60 units of amplitude and then samples will be centrifuged at 300 g for 5 min to remove cell debris. Supernatant will be used as AB homogenate after dilution to 10% in phosphate buffered saline and protease inhibitors.

Cell culture: Briefly, embryos (n=5 GD10 males, n=5 GD10 females, n=5 GD17 males, n=5 GD17 females) will be dissected and cortical regions removed using a standard embryonic mouse atlas (Schambra and Silver 2013). Tissues will be collected from the hippocampal ventricular zone and neocortex of the GD10 mice while hippocampal and neocortex tissue will be removed from GD17 mice (Schambra and Silver 2013). Tissue will be cultured as in previous experiments then plated on prepared coverslips for transfer to microfluidic chambers (Volpicelli-Daley et al. 2011; Tran et al. 2014).

Microfluidic Chambers: Three chambered microfluidic devices will be purchased (Xona Microfluidic, TCND500) and modified as follows. Using a well-defined protocol (see: Taylor et al. 2005) that has been modified to examine neuronal pathology (see: Tran et al. 2014) I will track the movement of AB proteins through axonal transport as it is propagated from chamber 1 to 3. In brief, each distinct chamber will be populated with approximately 100,000 neurons and grooves 10 µm wide and 3 µm in height will be made in the dividers between chambers to restrict their interactions to axonal growths. Importantly, a 75µl gradient between chambers 3 to 1 will maintain the flow of fluid from chamber 3 to chamber 1 (Tran et al. 2014). To ensure that AB homogenate added into chamber one will be unable to transfer into chambers 1 or 3 a control with empty chambers will be run. After inoculating chamber 1 with 2µl 10% AB homogenate (dosage based on: Morales et al. 2012 but may require alteration) samples will be taken from chambers 2 and 3 at 0.5, 1, 10, and 30 minutes and ran through a high sensitivity AB42 and AB40 ELISA (Millipore; Cat#EZBRAIN42, Cat#EZBRAIN40) to ensure no proteins introduced into chamber 1 interact with chamber 2 and 3 except by axonal transport.

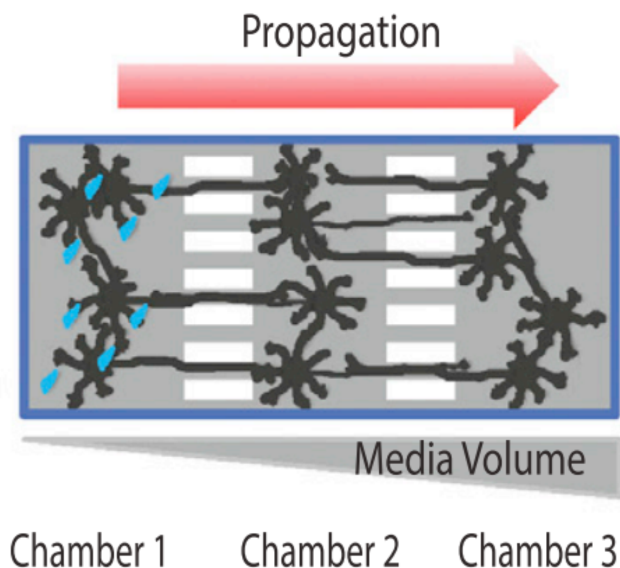

Figure 1: Three somal chambers filled with discrete neuron populations interrupted by microgrooves. The gradient of media volume will hold a constant flow from chamber 3 to 1. Blue arrowheads indicate the site of introduction of pathogenic AB homogenate (Modified from Tran et al., 2014)

Beta-Amyloid Imaging: A technique developed in 2002 by Klunk et al. showed great efficacy in the binding of 1,4-bis(4′-hydroxystyryl)-2-methoxybenzene (Methoxy-X04), a Congo Red derivative, to AB plaques for in vivo imaging. Their protocol can be easily adapted to the in vitro methods used here and the fluorescent nature of Methoxy-X04 will allow for easy quantification of AB deposits. Pictures will be taken on a Leica DMI6000 B microscope (Tran et al. 2014) directly from cell culture then the number of AB plaques/mm^2 determined using ScionImage (NIH). Greater density of amyloid plaques will indicate a larger AB burden.

Pilot study: A preliminary study will be run to confirm that inoculating HuAPPwt tissue with AB homogenate will lead to diseased pathology. For this pilot examination, two male and two

female setups will be developed from embryonic GD10 tissue. One male and one female setup will be inoculated with AB homogenate while the other will be inoculated with the vehicle. Images will be collected and analyzed on DI0, DI30, DI90 and at any other time points deemed necessary. This will not only be done to ensure that an increase in AB load is associated only with inoculation but also will help solidify the time points to examine the chambers in Aim 1. Paired-sample T tests will be used to examine differences between (i.e. DI0 female inoculated chamber 3 vs. DI30 female inoculated chamber 3). I expect that over time, accumulation of AB load will be seen only in AB homogenate inoculated setups.

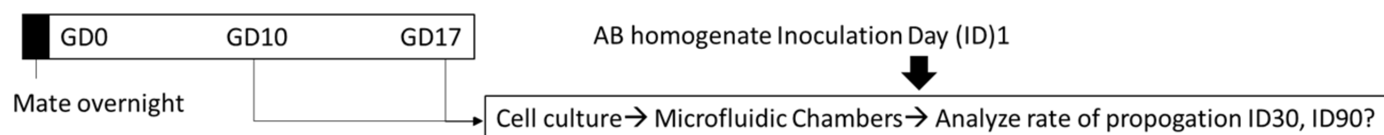

Figure 2: Methodology of Aim 1. Transgenic mice will be mated overnight and embryonic neural tissue collected on GD10 or GD17 then cultured. Glass slides will be inoculated with cultured cells and then affixed into a microfluidic chamber. The setup will isolate the extracellular environment of chamber 1 (see figure 1) from the other chambers allowing introduction of AB homogenate to only one population of neurons. Any detection of amyloid deposits in chambers 2 or 3 will indicate neuronal transmission of the pathology. The rate at which AB deposits into the other chambers will be analyzed at ID0, ID30 and ID90 (subject to alteration once data collection begins) to determine any sex differences in GD10 and GD17 tissue. Paired sample T tests will be used to analyze any differences, within sex and age groups, between ID0 and all other days. As I will collect tissue from two different brain regions, I will also run separate analyses on the different cell populations as well.

Predicted results: Firstly, just as it happens in vivo (Morales et al. 2012), I expect that there will be transfer of AB dysfunction from chamber 1 to 3 given enough time in both sexes and regions. Since the gradient prevents any transfer of AB between chambers except through axonal intervention, this will indicate neuron populations may infect those to which their axons are sent. How sex differences in the brain may lead to the different pathologies exhibited in humans (Lin and Doraiswamy 2015) and predicted to be found here is less understood. The little research that has been done on sex differences based on genetics at the cellular level indicate female cells are more sensitive to stressors leading to apoptosis (Penaloza et al. 2009). So, male brains may be protected by the presence of the y chromosome and given past studies on sex differences in AB load over time (Carroll et al. 2010; Jayaraman et al. 2012) it is likely this protection extends to AB pathology. As such, I expect to see lower rates of change from ID0 (not showing up until later time points i.e. ID90) in microfluidic chambers containing cells from male GD10 or GD17 mice. Still, it may be the first surges of testosterone that in some way impart a resistance to pathological spread of AB dysfunction. As testosterone is often converted into estrogen by aromatase to act upon the developing brain (McCarthy 2008), testosterone will also bring many of the benefits estrogen imparts (Simpkins et al. 2005; Simpkins et al. 2004). I therefore predict faster progression of disease pathology in GD10 males compared with their GD17 counterparts. Female GD10 and GD17 mice will likely have the largest and earliest change given the absence of hormones in their prenatal environment. In addition I will be looking at two regions, the hippocampus and neocortex. As estrogens are most protective within the hippocampus (Sherwin and Henry 2008) I expect that males will have lower rates of disease propagation in hippocampal cells compared with all females and with male neocortex tissue but only in the GD17 group.

Question 1

Possible issues: The gradient developed and used for the microfluidic chambers was used in experiments examining alpha-synuclein (Tran et al. 2014) so it may require alteration for use with AB. Since AB is around 4.5 kD and the gradient can be altered to prevent transfer of molecules as small as 0.2 kD (Taylor et al. 2005), this should not be a problem. Even if I do not find cell transfer through neuronal means, it would only indicate the need for other cell types. In order to facilitate this need, an in vivo study is appropriate and is therefore my next step. This in vivo study will not only provide this invaluable information, I will be experimentally altering hormonal levels during prenatal environments to establish their role in sex-biased Alzheimer pathology.

<u>Aim 2: Test the hypothesis that prenatal masculinization imparts a male-biased resistance to beta-amyloid cell to cell transmission and cognitive impairment in vivo.</u>

Animals: Female HuAPPwt mice will be placed overnight with male HuAPPwt (n=10 pairs) , once again the presence of a vaginal plug indicating GD 0. Females will then be injected once per day from GD12-17 with testosterone (n=10; 2.0µg of testosterone propionate (Sigma-Aldrich) in 0.5µl oil (Mann and Svare 1983)) or oil control (0.5µl n=10; 0.5µl). After birth, pups will be gonadectomized before postnatal day 17. Male mice (n=40) will be castrated and female (n=40) mice overiectomized to ensure no further hormonal interventions from the gonads during testing aside from those during prenatal exposure. A control group for surgery will not be included as this would introduce adult hormones as a variable.

Stereotactic injections: To induce Alzheimer like symptoms, which can be done at any age (Hamaguchi et al. 2011), stereotactic injections of 2 µl of 10% AB homogenate will be made into the prefrontal cortex (Paxinos and Franklin 2004) of gonadectomized 60 day old (adult) female and male mice born to a testosterone injected female (n=30 males; n=30 females) or oil injected female (n=30 males; n=30 females). A control group (n=10 castrated oil treated males n=10 overiectomized oil treated females) will be stereotaxically injected with 2 µl of a phosphate buffered saline with protease inhibitor.

Cognitive function: The cognitive function of mice given injections will be examined over several memory tasks. As cognitive function declines faster in females (Li and Singh 2014; Benice et al. 2006) I hope to determine if this mouse model displays this difference and examine the effect of prenatal hormonal environments. To test cognition a radial six arm water maze (RAWM) with a "Win-Stay" strategy will be used (Arendash et al. 2001). The RAWM has six equal length arms extending from a central region. After a learning trial where the mice are allowed to discover the location of the submerged platform, they will be placed back in the middle of the radial maze. Each time they make an error it will be marked down, the task will be to learn that each day the platform is moved to a new spot then remains in that location for that day ie "Win-Stay". As these mice will not present pathophysiology until around 10 months post injection, testing will be done between months 5-7 and 13-15 (Morales et al. 2012). The reduction in errors over subsequent trials will be used as a marker for cognition. If error rate increases over time between the two time periods it will indicate a loss of cognitive ability. Mean error rate will be analyzed with two-way-repeated-measures ANOVA with testing period as the within-subjects factor and treatment as the between subjects factor. Treatment groups will be as follows: testosterone treated males, testosterone treated females, oil treated males and oil treated females. Post-hoc least square differences will be used to analyze any significant interactions. Two way student T-tests will be used to ensure the AB homogenate injections were the cause of cognitive impairments by comparing treatment groups to vehicle injected animals.

Question 1

Quantification of pathology: At the end of month 15, half of the mice from each group will be anesthetized, perfused and brains will be sectioned at 30µm using a freezing, sliding microtome. Every third section will be kept for analysis. At month 20 the remaining mice will be similarly processed. Immunohistochemistry will be done to mark AB using BAM10 mouse anti-AB primary antibody (Abcam, AB7501) followed by biotinylated goat anti-mouse (Sigma), avidin conjugation and finally incubation in nickle-enhanced DAB (Puoliväli et al. 2002). Sections will be mounted, coverslipped and pictures taken of several regions of the brain. These will include the hippocampus, prefrontal cortex, entorhinal cortex, and cerebellum as a control region. Each section containing these will be photographed using a Nikon Coolpix990 camera and then altered to greyscale using Adobe photoshop CS6. Using ScionImage (NIH) the pictures will be analyzed for stained area fraction as a marker of AB burden (Amyloid area/mm^2). Separate ANOVA will be run on stained area fractions for each age group and each region using sex and treatment as variables (total of 8 separate ANOVAs). Analysis of vehicle injected tissue as suggested before will serve as confirmation of the pathological effects of the AB homogenate used.
Predicted Results: Firstly, I do not expect to see any cognitive impairments or increased level of amyloid load in the vehicle injected group. I expect to see the lowest spread of AB pathology and least attenuation of cognition in the males regardless of testosterone treatment. Then I would expect similar levels of AB pathology and loss of cognitive function in females treated with testosterone. Loss of cognition is expected to be greatest in females from oil treated dams along with the greatest spread of pathophysiology. Regional differences may also be present. Once again, given the protective nature of estrogens in the hippocampus (Sherwin and Henry 2008) I specifically expect less AB deposition in the hippocampus of testosterone treated animals compared with neocortex while oil treated females will have similar amounts within each region. Studying this paradigm in vitro with the microfluidic chambers would allow an evaluation of how much neurons contribute to the sex specific transport of pathogenic AB. Tissue could be collected on GD10, prior to prenatal hormonal surges, and cultured. Once placed into microfluidic chamber, male and female tissue would be subjected to a mimicked hormonal period with testosterone. Following a similar examination, the effect of feminization on male tissue will be determined.
Possible Issues: It may be the gain or gain and subsequent loss of sex steroid hormones in the female body that induce the greater level of pathology exhibited by their sex. Still, the findings from these proposed studies would be a valuable tool for understanding disease etiology regardless of the outcomes. Importantly, this study does not control for the male prenatal environment so any interpretations of the results will apply to sex differences and the female prenatal environment. Given that the burden of disease is greater in this population (Li and Singh 2014) and masculinization of tissue may lead to possible treatments, it is fitting to do this analysis first. Subsequent studies may inject pregnant females with flutamide, a potent anti-androgen, to examine how removing the effects of the prenatal hormonal surges (feminization) influence disease transmission in male tissues. Although I have opted to use surgery, it would be possible to do this with further genetic manipulations. Using an inducible-testicular feminization model or -estrogen receptor knock-out mice (Morris et al. 2005) would allow you to examine the effects of the first hormonal surges by blocking hormonal reception after they occur. Inducing the knock-out would then be an effective way to remove hormonal interventions from any steroids produced in the body. Still, the information gained from both aims will be vital for understanding sex differences in the abnormal cognitive impairments seen due to Alzheimer disease.

Question 1

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Student C – Pass

Reviewer 1:

This is well written and an extremely interesting proposal methodologically. Obtaining embryonic tissue from groups before and after the prenatal testicular surges to distinguish role of sex chromosomes vs early hormone exposure is creative. Some potential pitfalls are discussed and remedies for some of them are well thought out. It would have been ideal to more clearly indicate that the proposed methods have been used successfully before (90 day cell survival using microfluidic chambers). A potential problem in Aim 2 is related to the use of numerous siblings in the same experiment or apparently even in the same groups; student should be aware of statistical and interpretation confounds related to litter effects and there is a large literature on this topic (e.g., Holson, R. R., & Pearce, B. 1992 Neurotoxicology and Teratology, 14, 221-228). The justification for sacrificing some mice at 15 months and others at 20 months is unclear; some will be recently tested behaviorally and others not, and the statistical analyses proposed doesn't account for the difference in age.

Reviewer 2:

There is no title for this NRSA proposal.

The PI has chosen a very interesting topic. However, as little previous research has been done in this topic, the PI's proposal is mainly speculative and lacks scientific rationale. For example, it is not even clear whether there is any AD-related phenotypes in the proposed in vitro studies.

On page 1, it is stated: "I predict that masculinization of XX cells through….". What are the XX cells?

The significance section should describe the impact of your proposal rather than the importance of the problem.

The rationale of the proposed study is not adequately presented. Innovation does not equal to "has not been done previously", which is the minimal requirement of NIH proposals.

It is not clear whether the "pathological AB"-induced onset of pathology would occur in cultured neurons. It is not clear whether the detection of A-beta in chamber 2 and 3 are due to axonal transport or induced production of A-beta from the transgene (if it is not due to the APP transgene, then wild type neurons should also work for the proposed experiment).

It is not clear when the "after birth" gonadectomization" will be performed?

Reviewer 3:

Overall summary: This proposal aims to study how exposure to hormones prenatally can influence the cell-to-cell transfer of amyloid-β aggregates. Aim 1 will test how prenatal exposure to male hormones effects the subsequent distribution of Aβ in a primary neuron culture model paradigm. Aim2 will focus on how sex hormones can influence the spread of Aβ pathology and cognitive decline in an in vivo transgenic mouse model where exogenous human AD brain-derived Aβ will be intracerebrally injected in the brain. The focus of this proposal is on a significant topic (i.e. AD, sex differences and spread of cell pathology) and several aspects are innovative. Overall, this was a relatively well-written proposal that will focus on an interesting and innovative set of questions.

Significance: A pattern of cell-to-cell transfer has long been appreciated in AD, but the factors influencing this phenomenon and mechanisms by which this occurs are unclear and deserve attention. Thus, the significance of the problems being studied in this proposal is high and this is well described by the applicant.

Innovation: The idea that prenatal exposure to sex hormone may predispose neurons to enhanced/reduced susceptibility is novel and highly innovative. In addition, the use of the three chamber microfluidic devices is innovative and pertinent to addressing the spread of abnormal Aβ among neurons.

Approach:

Strengths – Use of microfluidic devices to study the cell-to-cell transfer of Aβ in vitro is a big strength of this proposal. In general, the approaches and methodologies are well described in this proposal.

Weaknesses – The hypotheses being tested in both aims 1 are not clearly communicated. It will be very difficult to attribute the effects observed in aim 2 to the Aβ within the human-derived samples that will be stereotaxically injected into the transgenic mice. Homogenates, like those described in the methods, will certainly contain several other proteins from the AD brain. How will the investigator know that the effects are solely from the Aβ in the sample and not other constituents of the lysate? The use of plaque load analyses may not work well in the culture model as it is not clear that Aβ plaques will be created in the culture system. Moreover, there is concern that neurons can be cultured for 90 days. Long-term primary neuron cultures are notoriously difficult and rarely (if ever) work well enough (without significant degeneration and glial expansion) to clearly interpret results.