

Author



After working in Professor Busciglio's lab for several months, Brandon Nguyen asked about the possibility of conducting a project independently. This project was attractive to him because it looks into connections between Alzheimer's disease and Down syndrome, something that had not been well studied to that point. Brandon was excited by the opportunity to break new ground and greatly appreciated the growth he achieved by being given the responsibility of acting as the project's lead researcher. Inspired by his experience, Brandon looks forward to pursuing more research opportunities through his education and future career.

Key Terms

- ◆ Age-Dependent Effects
- ◆ Calbindin Immunoreactivity
- ◆ Dp 16 Mouse Model
- ◆ Genetic Alterations of Calbindin, NPY, and Synaptoporin and Epilepsy
- ◆ Mossy Fiber Sprouting
- ◆ NPY Immunolabeling
- ◆ Seizure Activity

An Investigation on Epileptic Activity in a Transgenic Mouse Model of Down syndrome

Brandon T. Nguyen

Biological Sciences

Abstract

Down syndrome (DS) is the most common aneuploidy in humans, and is a leading cause of genetic mental retardation and learning disabilities in children. DS is closely linked to Alzheimer's disease (AD), as the majority of DS patients develop AD by 50 years of age. Previous studies have shown that patients with DS or AD have a higher incidence of seizures, but despite the correlations between seizures and dementia, the biological mechanisms influencing these processes are poorly understood. We used 12-month-old Dp16 mouse models of DS to identify markers of seizure activity in the hippocampus, a region of the brain that experiences neurodegeneration in AD. Markers were analyzed and included reduced expression of calbindin, increased expression of neuropeptide Y (NPY), and mossy fiber sprouting. Our results found increased expression of NPY, but no difference in calbindin or mossy fiber sprouting; consequently, it cannot be concluded that there is seizure activity in this mouse model of DS. As the results were not conclusive, more research will be required given that seizure activity may be age-dependent and may need to be explored in older animals. Another possibility is that alterations in gene expression in the trisomic mouse may alter the expression and activity of seizure-related proteins and signaling pathways. We are currently looking into these various alternatives.

Faculty Mentor



Faculty statement to come.

Jorge A. Busciglio

School of Biological Sciences

Introduction

Down syndrome (DS), the trisomy of chromosome 21, affects one in every 691 babies in the United States according to the National Down Syndrome Society (NDSS). Chromosome 21 carries the gene for amyloid precursor protein (APP) that produces the amyloid beta ($A\beta$) protein found in the neuritic (senile) plaques that are characteristic of Alzheimer's disease (AD). The prevalence of dementia in the institutionalized DS population is 8% between 35 and 49 years, 55% between 40 and 59 years, and 75% of those over 60 years old (Lai and Williams, 1989). Age-associated AD neuropathology is commonly found in individuals with DS because the trisomy of chromosome 21 increases the production of APP and the eventual amyloidogenic fragments and plaque formation. Both of these characteristics are consistent features in AD. In people with DS, soluble $A\beta$ oligomers, which result from the processing of the APP, appear in the brain decades before the extracellular deposition of plaques (Hirayama et al., 2003). Plaque formation causes axonal damage that may lead to the formation of neurofibrillary tangles or neuronal death through axonal flow disturbance and accumulation of $A\beta$ in cortical neurons (Hirayama et al., 2003).

DS and AD both have higher incidences of epilepsy than the general population. The incidence of epilepsy in persons with DS is 1.4–17% and varies with age, globally higher compared to the general population (~1%) (Barca et al., 2014). This value increases to over 46% in those over 50 (Romano et al., 1990). Seizures are a major area of interest in individuals with DS because those with new-onset seizures have greater cognitive decline (Lott et al., 2012). Seizures have been found to develop in 84% of individuals with DS and AD (Lai and Williams, 1989).

Electro-clinical findings of seizures in adults include myoclonic jerks on awakening and generalized spike and wave discharges on electroencephalogram (EEG) (Moller et al., 2001). Subclinical seizures (neuronal hyperactivity) are not readily observable and may not present any behavioral disturbances, but can cause biochemical and morphological alterations in the brain and contribute to neurodegeneration (Krsek et al., 2004; Cantalops and Routtenberg, 1996). Seizure-related activities affect language as well as reduction in autonomy, a greater risk of injury, and a higher mortality rate (Hommet et al., 2008).

Seizure-related cognitive decline is found in both AD and DS. The mechanisms relating seizures to cognitive decline in DS have not been elucidated, but high brain levels of

$A\beta$ are likely to be a factor (Lott et al., 2012). In hAPP (human APP) transgenic mice, $A\beta$ -induced epileptic activity was associated with sprouting of inhibitory axons in the molecular layer of the dentate gyrus (Palopp et al., 2007). However, due to the heterogeneity of seizures, the underlying cellular and molecular mechanisms that induce and propagate these abnormal electrical discharges in the brain remain poorly understood (Westmark et al., 2010). Identification of epileptic phenotypes in transgenic DS mice could provide models to aid in understanding of mechanisms underlying seizure in DS.

To study this, a Dp16 transgenic mouse model of DS was used. The genes on chromosome 21 in humans (Hsa21) are conserved on chromosomes 10, 16 and 17 of mice (Mmu10, 16, and 17). Each chromosome is trisomic for a unique subset of Hsa21 genes or their mouse orthologs (Gardiner, 2014). Many partial trisomy models have been developed to study this disease; however, the Dp16 mouse model was generated for the complete Hsa21 syntenic region on Mmu16 containing 110 orthologous genes (Li et al.) (Figure 1).

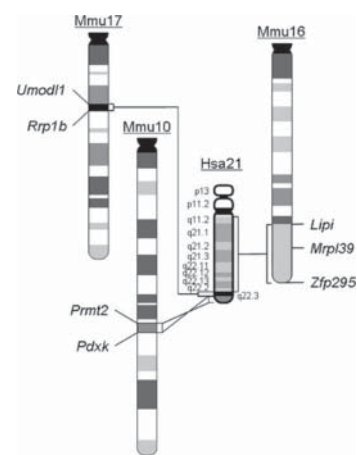


Figure 1

Chromosomal mapping of the mmu regions syntenic to Hsa21 (Adapted from Yu et al., Human Molecular Genetics, 2010).

Investigating the biological mechanisms in DS is valuable because this mouse model maps approximately 55% of the Hsa21 protein-coding genes (Gardiner, 2014). It must also be noted that the proteins assessed in this project (calbindin, neuropeptide Y (NPY), and synaptoporin) are located on different genes of the chromosome for humans and mice (Table 1).

Table 1

Gene Location of proteins of interest. The proteins of interest are not coded on the regions genetically altered in Dp16 transgenic mice.

Protein	Human	Mouse
Calbindin	8q21.3	4 5.55 cM
NPY	7pter-q22	6 24.04 cM; 6B3
Synaptoporin	3p14.2	14; 14A2

The hypothesis of this study is that the Dp16 transgenic mouse model of DS will experience neuronal hyperactivity. This will be determined by assessing the biochemical and morphological markers of seizure activity: reduced expression of calbindin, increased expression of NPY and mossy fiber sprouting.

Materials and Methods

Hippocampus Section Preparation

Six brain hemispheres of 12 month old transgenic Dp16 mouse models and aged-matched wild-type mice were fixed in 4% paraformaldehyde. Brains were sliced into 40 μ m sections with a vibratome and stored in PBS/NaN₃.

Immunohistochemistry

Hippocampal brain sections with the dentate gyrus and CA3 region (Figure 2) were selected for immunolabeling. Brain slices were transferred to wells with 500 μ L 1x PBS and washed twice with 500 μ L 1x PBS for 5 minutes each. The sections were blocked for 1 hour at room temperature with gentle rocking in 400 μ L 1x PBS/0.3% Triton-X/5% BSA/primary antibodies to reduce non-specific background staining. Afterwards, sections were incubated overnight at 4 °C with primary antibodies in 400 μ L 0.15% Triton-X/2.5% BSA for the proteins of interest (calbindin, NPY, and synaptoporphin) and MAP2 or TUJ1 as the neuronal marker. Calbindin and NPY were incubated at 1:1000 dilutions, while synaptoporphin was blocked at 1:750. MAP2 and TUJ1 were blocked at 1:6000 and 1:500 dilutions respectively. After primary antibody staining, brain sections were washed 3x for 5 minutes in 1x PBS. Sections were incubated in the dark for 1 hour at room temperature with 400 μ L 0.15% Triton-X/2.5% BSA/secondary antibodies at dilutions of 1:1000. Brain slices were washed 3x for 5 minutes in 1x PBS prior to being stained with Hoechst 1:10,000/1x PBS and later washed twice with 500 μ L of 1x PBS. Sections were mounted on Superfrost microscope slides with EMS Shield Mounting medium and dried for 1hr in an incubator.

Microscopy

The immunolabeled brain sections were imaged with an inverted Zeiss microscope using Axiovision software. The calbindin-labeled slices were imaged at 63x in two regions of the hilus of the dentate gyrus and two regions in the stratum radiatum for each subject. NPY-labeled sections were imaged at 63x in the CA3 region and synaptoporphin-labeled images were imaged at 63x in four regions of the stratum moleculare (Figure 2).

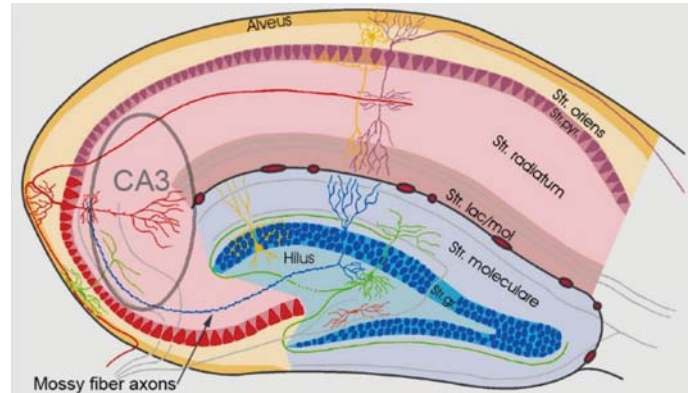


Figure 2

Hippocampal regions. NPY immunoreactivity was assessed in the CA3 region, synaptoporphin in the stratum moleculare, and calbindin immunoreactivity in the hilus was normalized to immunoreactivity in the stratum radiatum.

Analysis

Image J FIJI was used to auto-adjust image thresholds and calculate the percent area of immunolabeling of NPY in one field at 63x. Mossy fiber sprouting was assessed through immunolabeling for synaptoporphin, a synaptic protein highly expressed in the mossy fibers, and quantified with two methods, percent area at 63x and puncta count at 20x, in two to three fields of the molecular layer outside the granule cell layer of the dentate gyrus. The diffuse immunoreactivity of calbindin was calculated as described by Palop *et al.* (2010). Briefly, immunoreactivity in a control region (stratum radiatum) was used to normalize immunoreactivity in the hilus of the dentate gyrus of each subject at 63x in two fields of each region.

Results

Epileptic Activity of Wildtype vs Dp16

The hippocampus of six mice were assessed: three 12-month-old male Dp16 transgenic mice and three male wildtype. Each hippocampal section was labeled with Hoechst solution, MAP2, and one of the following; calbindin (Figure 3a, 3b), NPY (Figure 3c, 3d), and synaptoporphin (Figure 3e, 3f). The red staining represents the proteins of interest, and the green staining depicts the neuronal marker, MAP2, which identifies the somatodendritic compartment of the neuron or TUJ1, which is a soma-axonic marker.

Therefore, the results of transgenic Dp16 mice compared to wildtype mice showed a significant increase in NPY expression, but no significant change in calbindin expression or mossy fiber sprouting.

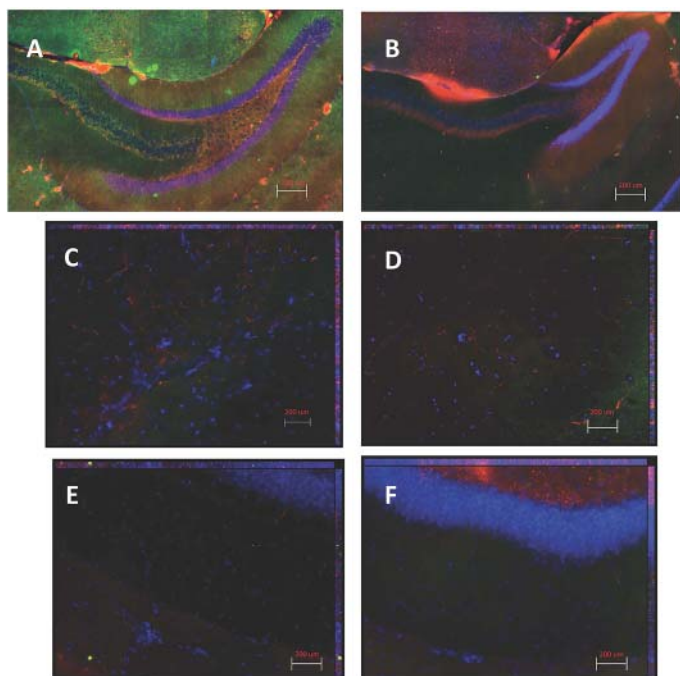


Figure 3

Immunolabeling for markers of seizure activity. A) Calbindin labeling in 12-month-old wildtype hippocampus. B) Calbindin labeling in 12-month-old Dp 16 hippocampus. Calbindin, red. C) CA3 region of 12-month-old male wildtype hippocampus. D) CA3 region of 12-month-old male Dp16 hippocampus. Calbindin, red; TUJ1, green. E) Molecular layer of the dentate gyrus in 12-month-old male wildtype hippocampus. F) Molecular layer of the dentate gyrus in 12-month-old male Dp16 hippocampus. MAP2, green; cell bodies, blue. Scale bar, 200 μ m. NPY expression (Figure 4a) was consistent with the expected results from sub-clinical seizure activity as shown by the increased immunolabeling in the CA3 region of the hippocampus of 12-month-old Dp16 transgenic mice. The normalized immunoreactivity of calbindin (Figure 4b) in the hilus of the dentate gyrus was not consistent with expected decreased calbindin expression as there was no significant alteration between the two subject groups. The percent area of synaptoporin (Figure 4c) also showed no change in the stratum moleculare.

Discussion

Electrophysiological Alterations in DS Individuals and Dp16 Mouse Model

Electroencephalogram (EEG) readings have shown that those with DS often have EEG abnormalities (Politoff et al., 1996). A study by Barca *et al.* (2014) found that 23% of patients with DS present with epilepsy, a value larger than found previously (~13–17%) (Bull and the Committee on Genetics, 2011; Thiel and Fowkes, 2004; Sangani et al., 2010; Ferlazzo et al., 2009; Hamouda et al., 2014; Ulate-Campos et al., 2014; Verotti et al., 2013).

Changes in synaptic plasticity have been reported in DS mouse models. Synaptic plasticity is measured as the ability of synapses to strengthen and weaken their communicative

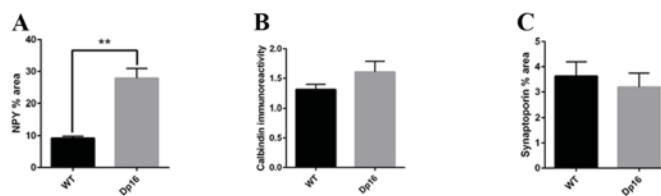


Figure 4

A) The percent area of NPY immunolabeling is increased in the CA3 region of the hippocampus of 12-month-old Dp16 transgenic mice. B) The normalized immunoreactivity of calbindin in the dentate gyrus is not altered, nor is the percent area of synaptoporin (C) in the molecular layer outside of the granule cell layer of the hippocampus. $**p < 0.01$, $n = 3$. Data were analyzed by two-tailed unpaired t test. Error bars indicate the mean \pm SEM. Calbindin, WT, 1.308 ± 0.09 ; Dp16, 1.608 ± 0.18 ; NPY, WT, 9.225 ± 0.61 ; Dp16, 27.89 ± 3.09 ; Synaptoporin, WT, 3.627 ± 0.57 ; Dp16, 3.192 ± 0.55 .

abilities between presynaptic and postsynaptic neurons over time. Long-term potentiation (LTP) is an electrophysiological phenomenon that describes the strengthening of synapses and is considered the correlate of learning and memory in the brain. The Dp16 model exhibits similar cognitive deficits to those with DS: impaired learning/memory and decreased hippocampal LTP (Yu et al., 2011). This has led to proposals that an imbalance of excitatory or inhibitory neurotransmission is responsible for changes in synaptic plasticity (Belichenko et al., 2004; Kleschevnikov et al., 2004; Hanson et al., 2007). Unbalanced neurotransmission can occur because the triplication of the Mmu16 may alter genetic expression levels of proteins that are associated with normal hippocampal synaptic plasticity. Further electrophysiological studies using EEGs should be performed to monitor electrical and phenotypic changes over a period of time. This can provide insight on the impact the Mmu16 syntenic region has on hippocampal synaptic plasticity at the physiological level. Confirming EEG abnormalities in Dp16 brains will allow for further investigation in the expression and activity of seizure related proteins (calbindin, NPY, and synaptoporin) and their respective signaling pathways.

Calbindin and Down syndrome

Calbindin is a critical calcium binding and buffering protein that has a major neuroprotective role in preventing neuronal death and maintaining calcium (Ca^{2+}) homeostasis. Altered levels of Ca^{2+} could disrupt homeostasis and impair proper calcium signaling, which leads to synaptic death and dysfunctional neuronal connections (Heizmann et al., 1992). It has also been found that calbindin blocks multiple pro-apoptotic pathways. For example, overexpression of calbindin inhibits pro-apoptotic events caused by $A\beta$ and presenilin-1 in glial and neuronal cells (Guo et al., 1998; Wernyj et al., 1999). With brains of AD patients shown to

have disrupted calcium homeostasis (Palotas et al., 2002; Foster and Kumar, 2002), and DS individuals experiencing cognitive decline at a younger age, analyzing the regulation of calbindin expression is a major point of interest when investigating the molecular mechanism behind early cognitive decline in individuals with DS.

This study's assessment of calbindin's immunoreactivity between the Dp16 model and the wildtype showed no significant alteration in the DG; however, previous research by Kobayashi *et al.* (1990) quantified calbindin expression with normal controls and clinically diagnosed patients with DS and found that the number and size of calbindin and parvalbumin immunoreactive neurons were significantly reduced in the cortex of patients with DS. These findings would suggest that neurons containing calbindin are affected by DS. Further studies into the signaling pathway of calbindin in subjects with Down syndrome would provide insight on the role of calbindin in seizure activity and dementia.

Calbindin Signaling Pathway, AD and DS

Recent findings have shown that calbindin also plays a critical role in AD pathogenesis. Calbindin buffers intracellular calcium, regulates neuronal synaptic function, and is produced in several cell types in the CNS (Baimbridge et al., 1992). Also, increased neuronal differentiation and neurite growth occurs when calbindin is overexpressed (Kim et al., 2006). Finally, calbindin appears to directly attach to caspase-3, which triggers early synaptic dysfunction in a Tg2576 AD mouse model (Bellido et al., 2002; D'Amelio et al., 2011). This evidence indicates calbindin is crucial for regulation of synapse formation, the basis of learning and memory.

The NMDA receptors, NR1 and two NR2 subunits, are molecules known to control memory function and synaptic plasticity (Nakanishi, 1992). In the brains of calbindin-KO AD mice, the protein levels of NR1 and NR2A decrease (Kook et al., 2014). Moreover, ERK1 and ERK2, which are both a part of the MAPK signaling pathway, rely on an influx of calcium from the NMDA receptor for activation. This is a key event in cognition through activation of CREB (Adams et al., 2002; Fukunaga et al., 1998; Bading et al., 1991). Kook *et al.* (2014) showed that when calbindin was removed, the activation of the ERK receptors and CREB decreased. These results suggested that knocking out calbindin could cause cognitive deficits and neuronal dysfunction.

Past research has added to a growing body of evidence that calbindin plays a significant molecular role in AD patholo-

gy and a potential critical role in DS during seizures. With DS individuals developing early-onset Alzheimer's disease (EOAD) and calbindin appearing to be a link between the two, studies in the calbindin signaling pathway in the Dp16 model will hopefully answer many questions.

NPY and Down syndrome

Neuropeptide Y acts as a neurotransmitter in the brain and has a neuroprotective role in the hippocampus. NPY positive cells account for 31% of GABAergic neurons in the hippocampus (Jinno and Kosaka, 2003). Normally, NPY will inhibit excitatory postsynaptic potentials (EPSPs) evoked in the CA1 and CA3 through presynaptic action, but not in dentate granule cells, and reduces the amplitude of excitation through hippocampal circuits (Klaptstein and Colmers, 1993; Haas et al. 1987). Without NPY, the brain is susceptible to seizure-induced damage. KA-induced seizures progress until death in 93% of NPY knock-out mice, while <20% in wild type littermates and intracerebroventricular infusion of NPY prevented seizure-related deaths (Baraban et al., 1997). Furthermore, hippocampal kindling increases expression of NPY in CA1, CA3, and the dentate gyrus (Bendotti et al., 1993; Schwarzer et al., 1995).

The results from NPY immunolabeling agreed with past results showing a significant increase of NPY expression in brains that could experience seizure activity; however, it is still unknown if the triplication of Mmu16 is responsible for the connection between DS and epileptic activity, which may increase the signaling pathway of NPY production for neuroprotection.

NPY Signaling Pathway, AD and DS

NPY is a key molecule involved in regulating the response of an organism to its external environment. It is very useful because of its plasticity, *i.e.* the capability to change its rate and level of expression. For example, a neuron's excitatory state in the hippocampus could change after seizures, which could be influenced by changing NPY levels (Scharfman and Gray, 2006). Extreme changes in NPY levels may be neurotoxic to the hippocampus and lead to eventual neuronal cell death. There are various NPY receptors (Y1, Y2, Y3, Y4, and Y5) throughout the dentate gyrus. Y2 and Y5 receptor proteins are found on the mossy fiber tract, while the Y1 receptor is located in the molecular layer (Kopp et al., 2002; Figure 5A).

The location of Y2 and Y5 indicates that these NPY receptors may play a role in presynaptic function, which is further supported by findings that suggests NPY influences mossy fiber transmission through a presynaptic mechanism

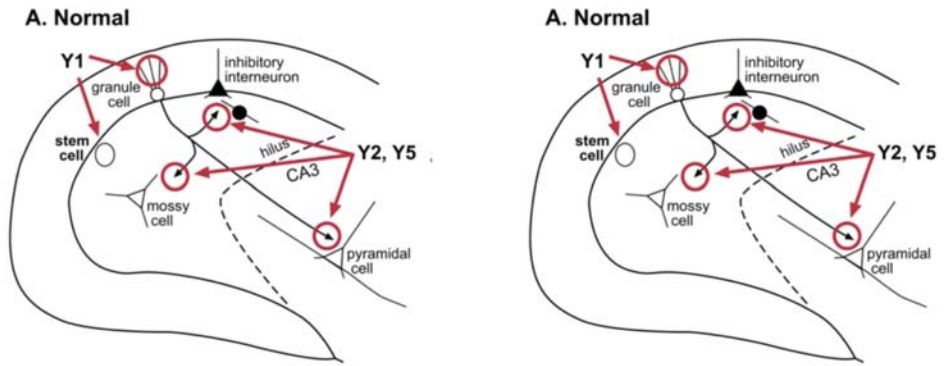


Figure 5

Alteration of NPY receptor expression in epilepsy. A) Y2 and Y5 receptor proteins are found on the mossy fiber tract, while the Y1 receptor is located in the molecular layer in non-epileptic hippocampus. B) The expression of Y2 receptors in the mossy fibers increases after both acute and chronic seizures. Adapted from Scharfman and Gray, *Experientia Supplementum*, 2006.

(Klapstein and Colmers, 1993; Guo et al., 2002; McQuiston et al., 1996).

In relation to seizure activity, NPY expression is elevated in response to status epilepticus, particularly in the hilus and granule cell layer (Scharfman and Gray, 2006). On the other hand, too much activity can cause NPY cells to die due to excitotoxicity (Mitchell et al., 1997). There is a clear distinction in the pattern of NPY depending if the seizures are acute or chronic. Acute seizures increase NPY expression in non-granule cells, but in chronic seizures, NPY is in non-granule cells and the granule cells and their axons. Moreover, there are major changes in NPY receptor expression after seizures (Figure 5B). The expression of Y2 receptors in the mossy fibers increases after both acute and chronic seizures, but not anywhere else (Sloviter, 1992). This finding is crucial because if individuals with Down syndrome are to experience electrical abnormalities, and there is an upregulation of NPY production, then there may also be an alteration of the gene that produces the Y2 receptor protein in the mossy fiber tract. If the Y2 receptor gene is changed in response to the development of the trisomic Dp16 mouse model, this could be an area of interest because NPY receptors may have therapeutic potential for anticonvulsants and prevent cognitive decline from neurotoxicity. Anti-epileptic treatments can be studied to target these receptors since they have a significant role in affecting overall cognitive function.

Synaptoporphin and Down syndrome

There are currently no reported investigations on synaptoporphin expression and Down syndrome.

Age-Dependent Effects in Dp16 Mice

There have been previous extensive behavior assessments of various mouse models of DS (Escorihuela et al., 1995; Coussons-Read and Crnic, 1996; Sago et al., 1998; Martinez-Cue et al., 2005; Costa et al., 2010; Garcia-Cerro et al., 2014). There is less insight on the neurological state of the Dp16 animals; however, Goodliffe et al. (2016), did a comprehensive analysis on the Dp16 mouse model. This study looked into the extent of neurological abnormalities in both young and adult mice. They

performed a number of tests to analyze behavior, motor function, and cognitive ability. It was found that Dp16 mice did not have deficits on the first day of acquisition, but exhibited a significant delay in late acquisition milestones after the second postnatal week. There were no changes in neurogenesis or cortical expansion in prenatal Dp16 mice, but a change in cortical cell population at developmental milestone periods. Neurological abnormalities were further studied in adult mice to see the long-range effects of the triplication of Mmu16. As expected, learning and memory tests on the Dp16 demonstrated cognitive impairment in adults. This study uncovered that the impairments became greater as the complexity of the tasks increased, which is consistent with past studies on individuals with DS (Campbell et al., 2013).

The results of this study could be taken a step further to study the cognitive abilities of aged Dp16 mice. As the results of our study were ambiguous, it may be necessary to study mice older than 12 months to identify seizure markers that could contribute to neuronal deficits. Since it has already been proven that the Dp16 mice experience age-dependent neuronal defects, the chances that there will be major impairments in aged mice are high. Seizure activity may be even more prevalent and noticeable in aged mice.

Conclusion

In our cohort of six mice, in which three were Dp16 and three were wildtype, our findings did not provide conclusive evidence that there are indications of seizure activity in the transgenic model of Down syndrome. Only NPY immunolabeling showed significant alterations from normal, while there was neither mossy fiber sprouting nor change in calbindin expression. Since recent studies have shown

that epilepsy is seen in those with both DS and AD, further research will be done given that there are other factors such as age dependency and alterations in gene expression that may affect the activity of these seizure markers and signaling pathways.

Better methods of characterization of epileptic phenotypes like EEG studies will be valuable in truly identifying signs of seizure activity. Thus, the goal is to investigate the molecular mechanism of neuronal hyperactivity behind this syndrome that leads cognitive deficits and hopefully discover better antiepileptic treatments that can prevent or slow down decline.

Acknowledgements

I would like to thank Dr. Vogler for her support, clear guidance, and positive encouragement. I would also like to thank Dr. Jorge Busciglio for allowing me to conduct research in his laboratory and for taking the time to patiently teach me. Finally, thank you to Dr. Kathleen Gardiner from the University of Colorado, who provided the mouse tissues used in this project.

Works Cited

- Adams JP, JD Sweatt. “Molecular psychology: roles for the ERK MAP kinase cascade in memory.” Annu Rev Pharmacol Toxicol 2002; 42: 135–163.
- Armon C, GW Peterson, BH Liwnicz “Alzheimer’s disease underlies some cases of complex partial status epilepticus.” J Clin Neurophysiol. 2000; 17:511–518. [PubMed: 1108555].
- Bading H, ME Greenberg. “Stimulation of protein tyrosine phosphorylation by NMDA receptor activation.” Science 1991; 253: 912–914.
- Baimbridge KG, MR Celio, JH Rogers. “Calcium-binding proteins in the nervous system.” Trends Neurosci 1992; 15: 303–308.
- Baraban SC, G Hollopeter, JC Erickson, PA Schwartzkroin, RD Palmiter. “Knock-out mice reveal a critical antiepileptic role for neuropeptide Y.” J Neurosci. 1997; 17(23):8927–36.
- Barca D, O Tarta-Arsene, A Dica, C Ilescu, M Budisteanu, C Motoescu, N Butoianu, D Craiu. “Intellectual Disability and Epilepsy in Down Syndrome.” MAEDICA—a Journal of Clinical Medicine 2015; 9(4): 344–350. [PubMed].
- Belichenko PV, E Masliah, AM Kleschevnikov, AJ Villar, CJ Epstein, A Salehi, WC Mobley. “Synaptic structural abnormalities in the Ts65Dn mouse model of Down syndrome.” Journal of Comparative Neurology, vol. 480, no. 3, pp. 281–298, 2004.
- Bellido T, M Huening, M Raval-Pandya, SC Manolagas, S Christakos. “Calbindin-D28k is expressed in osteoblastic cells and suppresses their apoptosis by inhibiting caspase-3 activity.” J Biol Chem 2000; 275: 26328–26332.
- Bendotti C, A Vezzani, G Tarizzo, R Samanin. “Increased expression of GAP43, somatostatin and neuropeptide Y mRNA in the hippocampus during development of hippocampal kindling in rats.” Eur J Neurosci. 1993 5(10):1312–20.
- Bull MJ and the Committee on Genetics—Clinical report—“Health supervision for children with Down syndrome.” Pediatrics 2011;128:393–406.
- Campbell C, O Landry, N Russo, H Flores, S Jacques, JA Burack (2013) “Cognitive flexibility among individuals with Down syndrome: assessing the influence of verbal and nonverbal abilities.” Am J Intellect Dev Disabil 118:193–200.
- Costa AC, MR Stasko, C Schmidt, MT Davisson (2010) “Behavioral validation of the Ts65Dn mouse model for Down syndrome of a genetic background free of the retinal degeneration mutation Pde6b(rd1).” Behav Brain Res 206:52–62.
- Coussons-Read ME, LS Crnic (1996) “Behavioral assessment of the Ts65Dn mouse, a model for Down syndrome: altered behavior in the elevated plus maze and open field.” Behav Genet 26:7–13.
- Escorihuela RM, A Fernandez-Teruel, IF Vallina, C Baamonde, MA Lumbreras, M Dierssen, A Tobena, J Florez (1995) “A behavioral assessment of Ts65Dn mice: a putative Down syndrome model.” Neurosci Lett 199: 143–146.
- Ferlazzo E, CK Adjien, R Guerrini, T Calarese, A Crespel, M Elia, P Striano, P Gelisse, P Bramanti, P di Bella, P Genton. “Lennox-Gastaut syndrome with late-onset and prominent reflex seizures in trisomy 21 patients.” Epilepsia 2009; 50:1587–95.
- Fukunaga K, E Miyamoto. “Role of MAP kinase in neurons.” Mol Neurobiol 1998; 16: 79–95.

- García-Cerro S, P Martínez, V Vidal, A Corrales, J Florez, R Vidal, N Rueda, ML ArbonesL, C Martínez-Cue (2014) "Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of Down syndrome." *PLoS One* 9:e106572.
- Goodliffe JW, JL Olmos-Serrano, NM Aziz, JLA Pennings, F Guedj, DW Bianchi, TF Haydar. "Absence of Prenatal Forebrain Defects in the Dp(16)1Yey/ Mouse Model of Down Syndrome." *Neurobiology of Disease* 2015; 36(10):2926–2944.
- Guo H, PA Castro, RD Palmiter, SC Baraban. "Y5 receptors mediate neuropeptide Y actions at excitatory synapses in area CA3 of the mouse hippocampus." *J Neurophysiol.* 2002; 87:558–566.
- Guo Q, S Christakos, N Robinson, MP Mattson. "Calbindin D28k blocks the proapoptotic actions of mutant presenilin 1: reduced oxidative stress and preserved mitochondrial function." *Proc Natl Acad Sci USA* 1998; 95: 3227–3232.
- Hamouda HB, H Mnasri, S Ghanmi, H Soua, G Sakli, M Dogui, MT Sfar. "West syndrome associated with Down syndrome: Case report and literature review." *Pediatrics Polska.* 2014;89:203–6.
- Hanson JE, M Blank, RA Valenzuela, CC Garner, DV Madison. "The functional nature of synaptic circuitry is altered in area CA3 of the hippocampus in a mouse model of Down's syndrome." *Journal of Physiology*, vol. 579, no. 1, pp. 53–67, 2007.
- Heizmann CW, K Braun. "Changes in Ca(2+)-binding proteins in human neurodegenerative disorders." *Trends Neurosci* 1992; 15: 259–264.
- Hirayama A, Y Horikoshi, M Maeda, M Ito, S Takashima. "Characteristic developmental expression of amyloid beta40,42, and 43 in patients with Down syndrome." *Brain Dev* 2003; 25:180–5.
- Hommet C, K Mondon, V Camus, B De Toffol, T Constans. "Epilepsy and dementia in the elderly." *Dement Geriatr Cogn Disord.* 2008; 25:293–300. [PubMed: 18311076].
- Jinno S, T Kosaka. "Patterns of expression of neuropeptides in GABAergic nonprincipal neurons in the mouse hippocampus: Quantitative analysis with optical dissector." *J Comp Neurol.* 2003 461(3):333–49.
- Klapstein GJ, WF Colmers. "On the sites of presynaptic inhibition by neuropeptide Y in rat hippocampus in vitro." *Hippocampus.* 1993;3:103–111.
- Kleschevnicov AM, PV Belichenko, AJ Villar, CJ Epstein, RC Malenka, WC Mobley. "Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of down syndrome." *Journal of Neuroscience*, vol. 24, no. 37, pp. 8153–8160, 2004.
- Koffie, RM, BT Hyman, TL Spires-Jones. 2011. "Alzheimer's disease: synapses gone cold." *Mol. Neurodegener.* 6, 63.
- Kook S-Y, H Jeong, MJ Kang, R Park, HJ Shin, S-H Han, SM Son, H Song, SH Baik, M Moon, EC Yi, D Hwang. "Crucial role of calbindin-D28k in the pathogenesis of Alzheimer's disease mouse model." *Nature.* 2014;21:1575–1587.
- Lai F, RS Williams. "A prospective study of Alzheimer's disease in Down syndrome." *Arch Neurol* 1989; 46:849–53.
- Lott I, E. Doran, V Nguyen, A Tournay, N Movsesyan, D Gillen. "Down Syndrome and Dementia: Seizures and Cognitive Decline." *J Alzheimer's Dis.* 2012 ; 29(1): 177–185.
- Martínez-Cue' C, N Rueda, E García, MT Davisson, Schmidt C, Flo' rez J (2005) "Behavioral, cognitive and biochemical responses to different environmental conditions in male Ts65Dn mice, a model of Down syndrome." *Behav Brain Res* 163:174 –185.
- McQuiston AR, JJ Petrozzino, JA Connor, WF Colmers. "Neuropeptide Y1 receptors inhibit N-type calcium currents and reduce transient calcium increases in rat dentate granule cells." *Neurosci.* 1996; 16:1422–1429.
- Mendez M, G Lim. "Seizures in elderly patients with dementia: Epidemiology and management." *Drugs Aging.* 2003; 20:791–803. [PubMed: 12964886].
- Menéndez M. "Down syndrome, Alzheimer's disease and seizures." *Brain Dev.* 2005; 27:246–252. [PubMed: 15862185].
- Moller JC, HM Hamer, WH Oertel, F Rosenow. "Late-onset myoclonic epilepsy in Down's Syndrome (LOMEDS)." *Seizure.* 2001; 10:303–306. [PubMed: 11466028].
- Nakanishi S. "Molecular diversity of glutamate receptors and implications for brain function." *Science* 1992; 258: 597–603.

- Palop JJ, J Chin, ED Roberson, J Wang, MT Thwin, N BienLy, J Yoo, KO Ho, GQ Yu, A Kreitzer, S Finkbeiner, JL Noebels, L Mucke. "Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease." *Neuron*. 2007; 55:697–711. [PubMed: 17785178].
- Palop JJ, B Jones, L Kekonius, J Chin, GQ Yu, J Raber, E Masliah, L Mucke. "Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits." *Proc Natl Acad Sci U S A*. 2003; 100:9572–9577.
- Palotas A, J Kalman, M Palotas, A Juhasz, Z Janka, B Penke. "Fibroblasts and lymphocytes from Alzheimer patients are resistant to beta-amyloid-induced increase in the intracellular calcium concentration." *Prog Neuropsychopharmacol Biol Psychiatry* 2002; 26: 971–974.
- Politoff AL, RP Stadter, N Monson, P Hass. "Cognition-related EEG abnormalities in nondemented Down syndrome subjects." *Dementia*. 1996;7:69–75.
- Puri BK, KW Ho, I Singh. "Age of seizure onset in adults with Down's syndrome." *Int J Clin Pract* 2001; 55:442–4.
- Sangani M, A Shahid, S Amina, M Koubeissi. "Improvement of myoclonic epilepsy in Down syndrome treated with levetiracetam." *Epileptic Disord*. 2010; 12:151–4.
- Sago H, EJ Carlson, DJ Smith, J Kilbridge, EM Rubin, WC Mobley, CJ Epstein, TT Huang (1998) "Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities." *Proc Natl Acad Sci U S A* 95:6256–6261.
- Scharfman HE, WP Gray. "Plasticity of neuropeptide Y in the dentate gyrus after seizures and its relevance to seizure-induced neurogenesis." *EXS*.2006;(95):193–211.
- Schwarzer C, JM Williamson, EW Lothman, A Vezzani, G Sperk. "Somatostatin, neuropeptide Y, neurokinin B, and cholecystokinin immunoreactivity in two chronic models of temporal lobe epilepsy." *Neuroscience*. 1995; 69(3):831–45.
- Thiel RJ, SW Fowkes. "Down syndrome and epilepsy: a nutritional connection?" *Med Hypotheses*. 2004;62:35–44.
- Ulate-Campoas A, A Nascimento, C Ortez. "Down's syndrome and epilepsy." *Rev Med Int Sindr Down* 2014;18:3–8.
- Verotti A, R Cusmai, F Nicita, A Pizzolorusso, M Elia, N Zamponi, E Cesaroni, T Granata, I De Giorgi, L Giordano, S Grosso, P Pavone, E Franzoni, G Coppola, C Cerminara, P Curatolo, S Savasta, P Striano, P Parisi, A Romeo, A Spalice. "Electroclinical features and long-term outcome of cryptogenic epilepsy in children with Down syndrome." *J Pediatr*. 2013;163:1754–8.
- Wernyj RP, MP Mattson, Christakos S. "Expression of calbindin-D28k in C6 glial cells stabilizes intracellular calcium levels and protects against apoptosis induced by calcium ionophore and amyloid beta-peptide." *Brain Res Mol Brain Res* 1999; 64: 69–79.
- Westmark CJ, PR Westmark, JS Malter. "Alzheimer's Disease and Down Syndrome Rodent Model Exhibit Audiogenic Seizures." *J Alzheimers Dis*. 2010 ;20(4): 1009–1013. doi:10.3233/JAD-2010-100087.
- Yu T, Z Li, Z Jia, SJ Clapcote, C Liu, S Li, S Asrar, A Pao, R Chen, N Fan, S Carattini-Rivera, AR Bechard, S Spring, RM Henkelman, G Stoica, SI Matsui, NJ Nowak, JC Roder, C Chen, A Bradley, YE Yu. "A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions." *Hum Mol Genet*. 2010 19(14): 2780–2791.

