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Parallels Between Down Syndrome and Alzheimer's Disease

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Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Parallels between Down Syndrome and Alzheimer's Disease

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Department Psychology

Abstract
Down syndrome is caused by trisomy of human chromosome 21 (Hsa21) and often leads to Alzheimer's disease in affected individuals. The Tc1 mouse model contains a copy of Hsa21 in its genome, and is therefore trisomic for the genes it contains to manifest as a Down syndrome mouse model. Transmission of Hsa21 to offspring generations occurs at a low frequency with fertility rates decreased in comparison to control mouse lines.
This experiment has verified that presence of Hsa21 causes a significant increase from normal brain weight. Hsa21 carriers demonstrated reductions in the number and length of dendritic projections and the number spines on cortical neurons suggesting that the increase in brain size may have been due to changes in glial cell proliferation rather than an increase in gray matter. Some have reported increases in beta-amyloid plaques in affected persons which can induce gliosis. These observations have led us to begin a study to verify the presence of beta-amyloid plaques and cell density to better understand the changes Hsa21 produces in the brain. Despite relatively extreme pathological changes in the brain the behavior of Hsa21 carriers did not exhibit significant memory impairments as compared to controls. There was a trend towards memory impairment. However, some of the subjects appeared to be hypoactive (a trait associated with Down Syndrome) that may have confounded our test that was dependent on normal motor function. Studies of spontaneous activity are now underway.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature _____ Date _____

Faculty Sponsor Signature _____ Date _____

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Abstract:

Down syndrome is caused by trisomy of human chromosome 21 (Hsa21) and often leads to Alzheimer's disease in affected individuals. The Tc1 mouse model contains a copy of Hsa21 in its genome, and is therefore trisomic for the genes it contains to manifest as a Down syndrome mouse model. Transmission of Hsa21 to offspring generations occurs at a low frequency with fertility rates decreased in comparison to control mouse lines.

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Purpose:

Down syndrome (DS) encompasses the majority of live born chromosomal abnormalities and results in cognitive impairment along with other debilitating health problems (Gardiner et al, 2010). DS is caused by trisomy, or three copies versus the normal two, of chromosome 21

(Hsa21). People with DS exhibit impaired ability to put words together, poor ability to recall events or objects (explicit long-term memory), as well as deficient verbal short-term memory.

A feature frequently seen in DS is the presence of Alzheimer's (AD) symptoms of memory impairment and neuropathy, including the presence of neurofibrillatory tangles and beta-amyloid plaques in the brains of 50% of people with DS at thirty years of age (Rueda et al, 2010). Interestingly, both DS and AD are influenced by chromosome 21, which not only causes DS when in a trisomy, but also contains an extra copy of the gene for the amyloid-precursor protein (APP) (Palmer, 2011). The subsequent overexpression of the APP gene leads to an increased risk for AD in people with DS. As neuronal damage from AD neuropathy is irreversible, it is vital to find and stop the damage from AD in DS as quickly as possible.

The overlap in pathology between people with AD and people with DS suggests that this is where the current research on DS should be directed. Studies in this area could open doors to improving cognitive abilities in people with DS, and subsequently, extend and improve their overall quality of life. The exploration of the present study is focused on this goal.

The Tc1 mouse model of DS from Jackson Laboratories offers an excellent opportunity to explore prenatal aspects in DS. These mice are genetically modified to produce offspring with DS (O'Doherty et al, 2005). The main advantage of using the Tc1 mouse is that the first 21 days postnatal corresponds with the developmental cycle of a human still *in utero* (Sherwood and Timiras, 1970). Utilizing this correlation between human and rodent development allows us to directly observe brain development for comparison to stages of human *in utero* development, and identify early behavioral and brain development changes and potential therapeutic interventions. Therefore, using this particular mouse model to try to identify underlying causes and possible

routes for further investigation is nearly ideal in the study of Down syndrome. Therefore, the purpose of this experiment was to compare neurological and behavioral similarities between Down syndrome to those of Alzheimer's disease using a mouse model of DS.

Research Design:

Validation of the mouse model

In this the first part of this experiment, a mouse model for DS, the B6129S-Tc(Hsa21)1TybEmcf/J from Jackson Laboratories, was compared with non-diseased control mice (mouse model C-57 BL/6J) (The Jackson Laboratory, Bar Harbor, ME). The Tc1 model is a transchromosomic mouse that carries an extra copy of human chromosome 21 (Hsa21) (O'Doherty et al 2005). As compared to other mouse models for DS that contain trisomy of mouse chromosomes, the Tc1 model expresses trisomy exclusively for the genes on the inserted chromosome, Hsa21. O'Doherty *et al* created this transchomosomic model by inserting approximately 90% of Hsa21 into mouse embryonic stem cells, resulting in a fairly stable and transmittable germline exhibiting chimeric features also seen in human DS mosaicism (both trisomic and normal cells exist in the same specimen).

In the first phase of this experiment, Tc1 mice were put through a longitudinal study to evaluate learning and memory. These tests were completed at 25 days, 42 days, 90 days, 12 months, and 24 months and consisted of training for the step-down test for inhibitory avoidance/learning followed by a 24 hour and 7 day memory retention test (using the methods of Izquierdo, Fiorenza, Rosa, Myskiw; 2012). Additional tests completed at 90 days including the open-field and dark-light tests. All of the tests were compared to age matched control mice. Two female Hsa21 positive female breeders and their mates were tested at one year of age with the

step-down test of test of inhibitory avoidance (training, 24 hour retest, and 7 day retest), open-field, dark-light, and spontaneous alteration t-maze to assess hippocampal functioning (using the methods of Gerlai, 1998).

Along with behavior tests the neuropathy of the Tc1 model was addressed. After the 90 day testing was completed, mice were euthanized and sample of brain, tissue from the tail and ear (for genotyping), and blood samples were obtained. Genotyping via polymerase chain reaction (PCR) and gel electrophoresis was completed to identify mice that were carriers of Hsa21. Histology work for AD pathology, represented by beta-amyloid plaques and tangles, reduced cell count, and lowered spine numbers were completed. These stains consisted of Bielschowsky's stain (beta-amyloid plaques), cresyl violet (cell density), and golgi-thionin (dendrite size and counting the number dendritic spines).

Results:

Identification of the transgene at 205 base pairs (bp) was completed using PCR (see: Figure 1). An internal positive control band at 324 bp needed to be present in all subjects to be considered a valid PCR result. From Figure 1 it can be seen that subject DS07 carries the transgene, Hsa21, and is therefore a positive carrier. The rest of the subjects in the sample gel were negative for the Hsa21 transgene. Out of the 18 offspring of Tc1 breeders, three mice were found to be positive carriers for Hsa21. Reproduction rates overall were dramatically lower (1 to 6 mice per litter) than most mouse strains (8-16) in our colony. PCR and gel electrophoresis were completed for all subjects. Tc1 results are shown in Table 1 along with relative brain weights as compared to body weight on the day of sacrifice. Golgi-thionin staining revealed

dramatic decreases in both the number and length of dendrites in the cortex as well as the number of spines in the Hsa21 carriers compared to noncarriers, (see Figure 2).

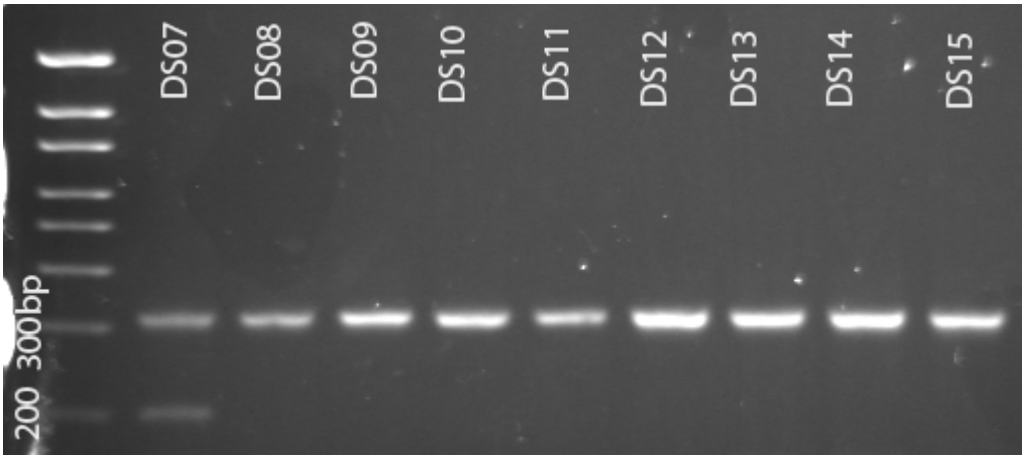

Figure 1. Sample of gel electrophoresis of PCR products. In this example only DS07 carries the transgene Hsa21.

Behavioral data for the step-down test of inhibitory avoidance beginning at 25 days of age for subjects DS07-DS24 and through 90 days of age is shown in Table 2. Breeders DS28-DS30 were trained at one year of age and completed a 24 hour and 7 day retest. The step-down latency at each time point was found by subtracting the initial training time from the time at data points from 24 hour, 7 day, 42 day, and 90 day retests. The average step-down latency of Hsa21 carriers and noncarriers (see Table 1) was calculated and (see Figure 3). The mean number of boli (an index of emotionality) at each test point was also compared between Hsa21 carriers and noncarriers (see Figure 4).

Table 1. Genotyping results and relative brain weights for Tc1 subjects. The average relative brain weight of Hsa21 carriers (20.12 g) was significantly greater than that of Hsa21 negative mice (16.23 g) with $t(20) = 3.282$, $p < .05$.

Code	Hsa21	Age	Gender	Relative Brain Weight
DS07	+	90 d	f	19.86
DS08	-	90 d	f	17.85
DS09	-	90 d	f	19.00
DS10	-	90 d	m	15.59
DS11	-	90 d	m	14.13
DS12	-	90 d	f	19.78
DS13	-	90 d	m	15.21
DS14	-	90 d	m	11.20
DS15	-	90 d	m	15.19
DS16	-	90 d	m	14.87
DS17	+	90 d	f	22.94
DS18	-	90 d	m	14.07
DS19	-	90 d	m	13.78
DS20	+	90 d	f	21.46
DS21	-	90 d	f	19.25
DS22	-	90 d	f	19.35
DS23	-	90 d	f	17.31
DS24	-	90 d	f	17.70
DS27	+	1 yr	f	17.69
DS28	+	1 yr	f	18.67
DS29	-	1 yr	m	16.39
DS30	-	1 yr	m	15.32

$$relative\ brain\ weight = \frac{brain\ weight\ (g)}{body\ weight\ (g)} * 1000$$

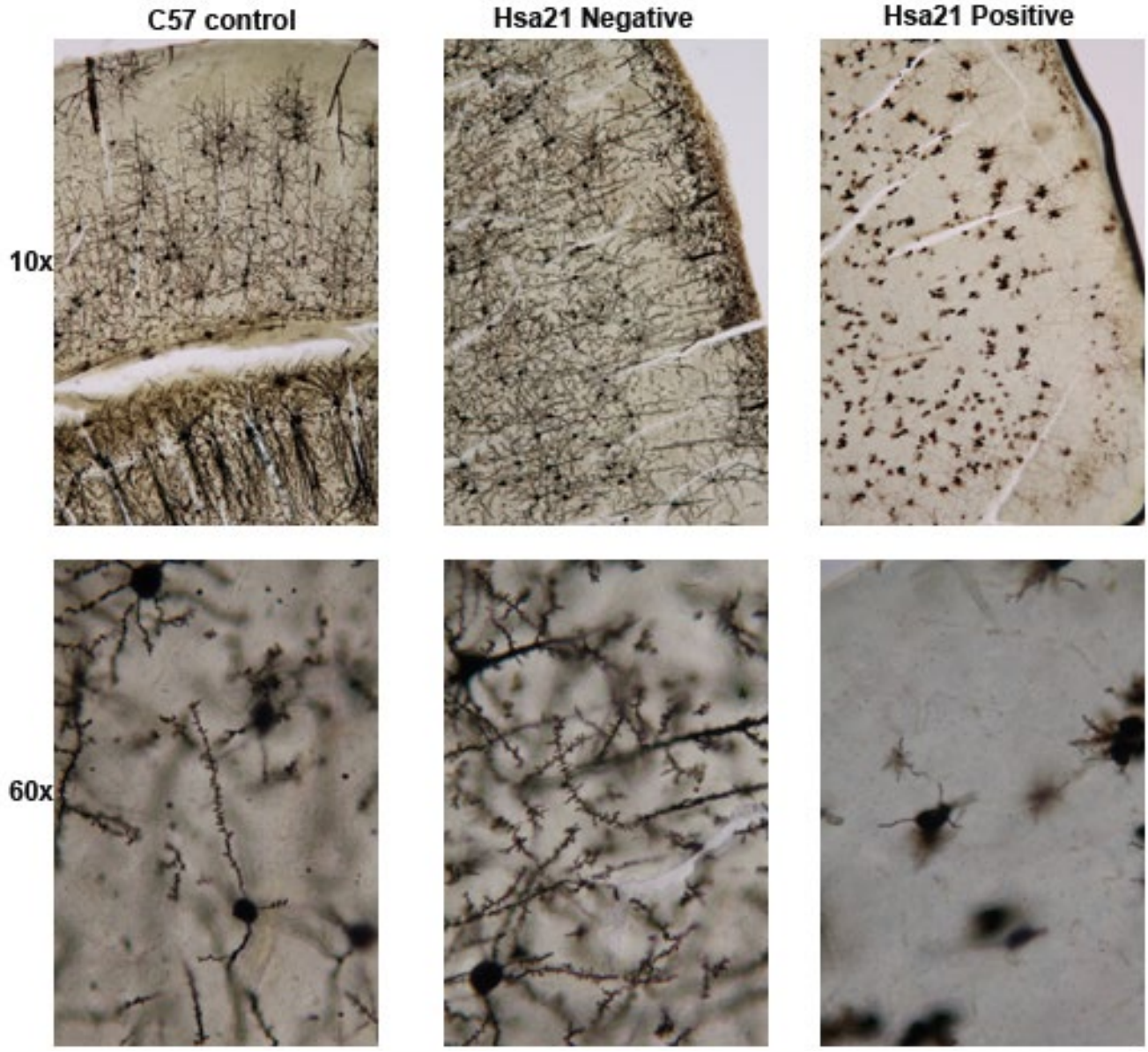

Figure 2. Golgi thionin staining of neurons in a control, Tc1 control, and Tc1 Hsa21 carrier. The Hsa21 carrier demonstrates somas in the cortex, but extensively reduced axon projections, as compared to Hsa21 negative subjects and control.

Table 2. Raw data from step-down test of inhibitory avoidance learning.

Code	Training		24 hr retest		7 d retest		42 day retest		90 day retest	
	time (s)	boli	time (s)	boli	time (s)	boli	time (s)	boli	time (s)	boli
DS07	36.88	0	300.00	5	99.09	2	166.1	3	300	4
DS08	13.78	0	148.41	0	270.09	2	81	1	300	3
DS09	11.72	0	11.59	0	48.01	0	75	1	39.03	2
DS10	186	1	300.00	1	300	4	215	2	178.25	1
DS11	10.85	0	28.00	0	108.4	2	259	4	231.6	3
DS12	17.47	0	42.46	1	79.88	1	32.81	0	57.25	0
DS13	21.53	0	39.00	0	300	1	300	1	286.19	2
DS14	44.44	1	300.00	1	300	1	200.34	1	300	3
DS15	29.08	0	268.62	4	187.6	1	74.85	1	78.22	2
DS16	266.47	0	300.00	1	300	3	300	5	300	3
DS17	49.31	0	99.63	1	53.31	1	27.62	1	23.13	0
DS18	70.47	1	300.00	2	300	0	300	3	300	5
DS19	14.04	0	183.88	2	106.88	1	259.57	1	219.65	3
DS20	36.58	0	111.87	3	121.5	0	241.94	1	234.84	4
DS21	29.31	0	99.83	2	99.8	0	113.09	1	97.06	1
DS22	64.18	0	101.00	1	60.31	0	262.63	2	300	6
DS23	16.12	2	8.96	0	151.15	0	91.75	1	283.19	3
DS24	21.78	0	243.22	1	138.97	0	300	5	300	2
DS27	5.00	0	300.00	3	300.00	6	-	-	-	-
DS28	57.60	0	202.47	0	16.84	0	-	-	-	-
DS29	6.30	0	300.00	6	300.00	7	-	-	-	-
DS30	6.10	0	300.00	3	300.00	2	-	-	-	-

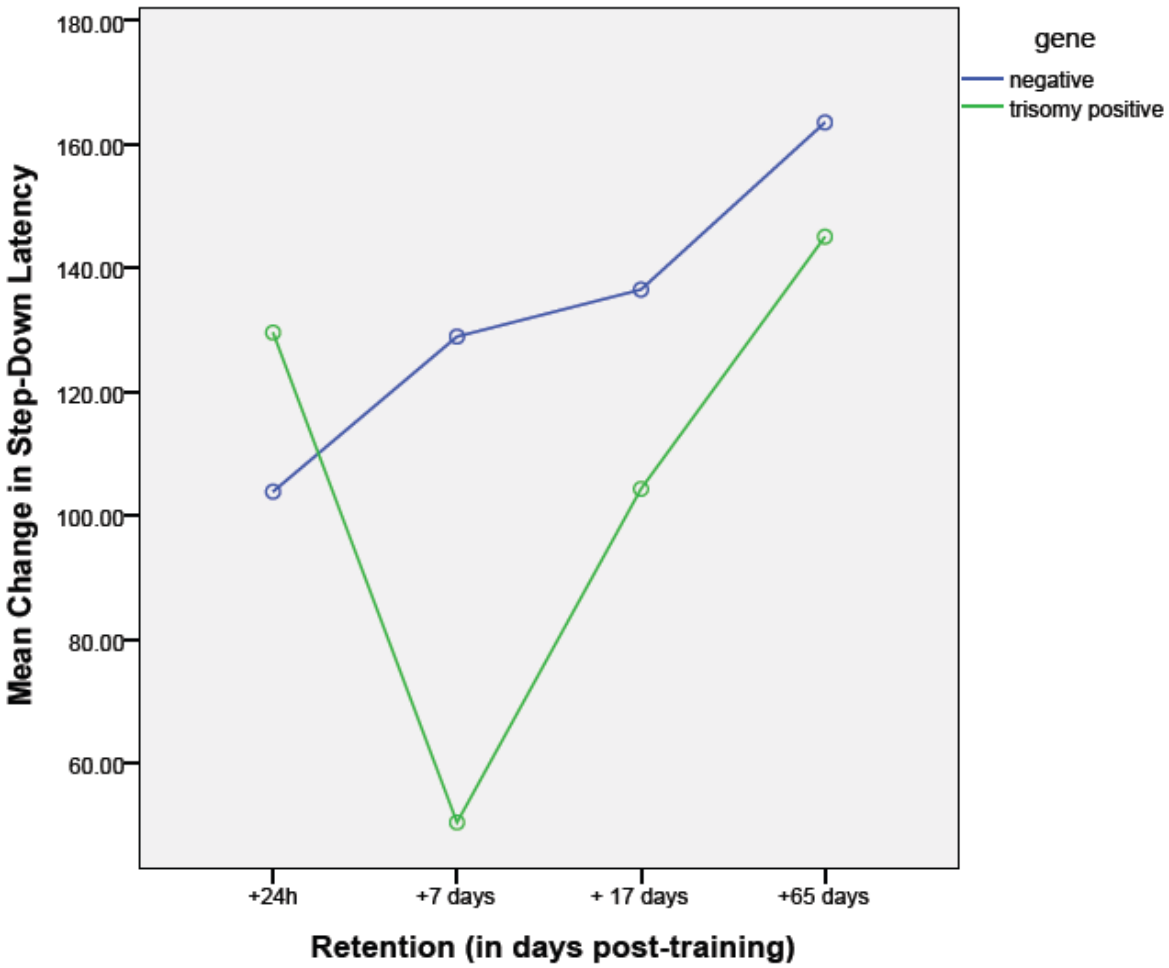

Figure 3. Step down latency adjusted from initial training step down time. Hypomotor activity may have influenced increased step down latency in Hsa21 positive subjects during the 24 hour retest period. Trends towards memory impairment of Hsa21 carriers as compared to noncarriers were seen throughout the rest of the test points.

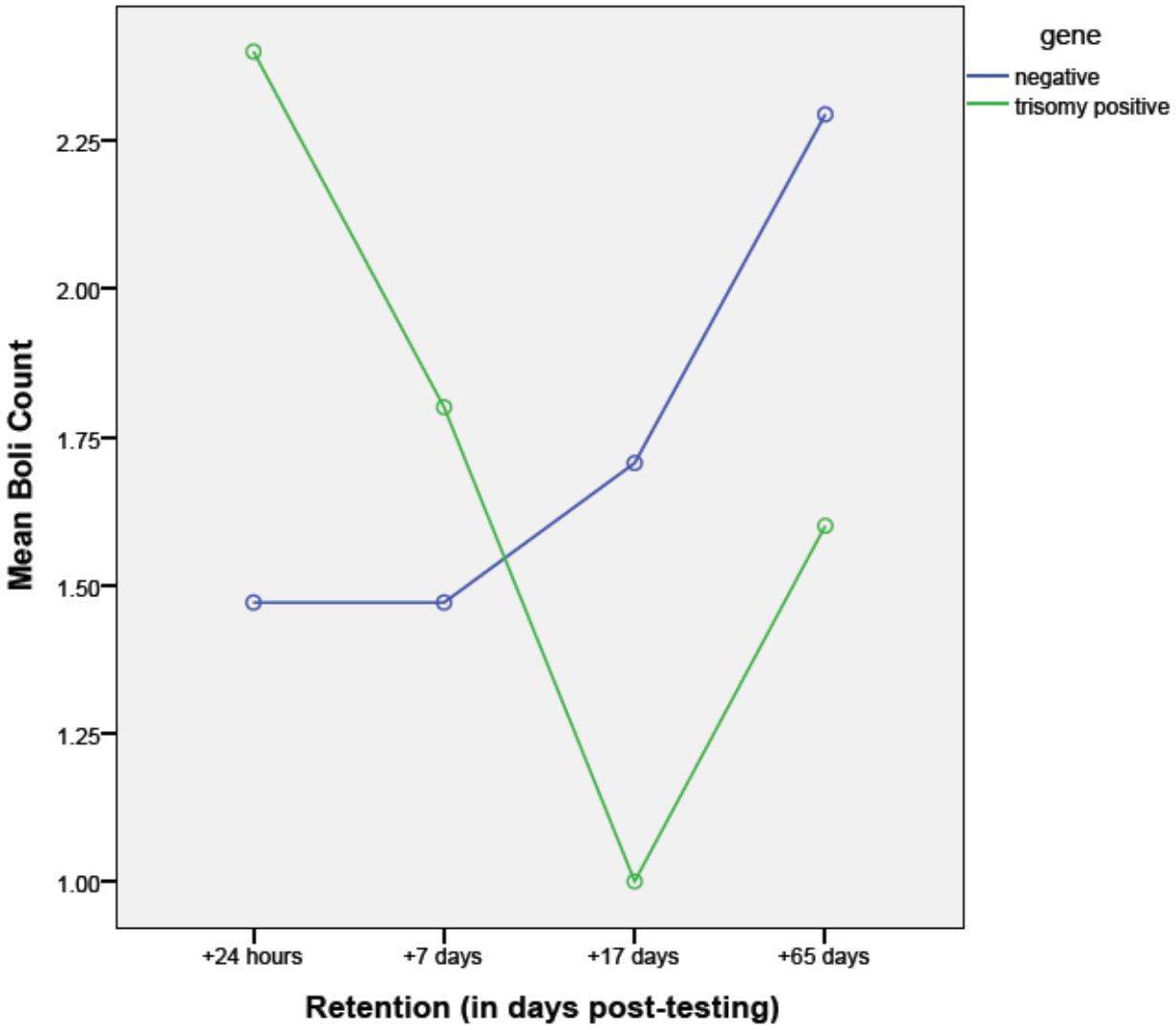

Figure 4. Mean number of boli at each test point for Hsa21 noncarriers (negative) and carriers (trisomy positive). 24 hour and 7 day retest data indicates increased anxiety in Hsa21 carriers as compared to noncarriers. This trend was reversed in the remaining test points.

Discussion:

This study has successfully demonstrated that the Hsa21 gene does get passed on to offspring generations in mice in a way that is similar to human Down syndrome. However, the transmission rate of the gene was very low (16.7%) and fertility rates of the Tc1 females were also lower than other mouse strains. The profound decrease in dendrite length and projections in

the brain (see Figure 2) suggests that addition of Hsa21 into the mouse genome produces severe neurological effects consistent with human Down syndrome. This observation is further supported by statistically significant changes in relative brain weight of Hsa21 positive mice compared to controls.

Although trends in behavioral data were noted (see Figures 3 and 4), the observations were not statistically significant. This is not surprising given our difficulty in breeding large numbers of Hsa21 positive subjects for testing. The number of positive subjects (N=5) compared to controls (N=18) limited the statistical power of our tests. For this reason we plan to obtain new breeders in August of 2013 and continue the project beyond the grant period. We feel this is justified given the very promising changes noted in the brain and their similarity to the human form of Down syndrome. In addition, we also suspect that our use of an activity dependent memory test may have skewed the behavioral data. Since evidence of memory was scored as an increase in immobility on the test platform (failing to step down) any reduction in activity levels would have appeared as better retention. We have completed tests of activity (open-field) and anxiety (light-dark test) and are in the process of training scorers to analyze the data. We have also completed staining with cresyl violet (to verify neuron numbers) and a beta-amyloid staining. We plan to score these slides next fall. The data from these studies will be presented at the annual Midbrains Undergraduate Research Conference in October 2013.

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