Title:	**Behavioral phenotyping of the Ts65Dn mouse model for Down syndrome**

Procedures	**Delayed Matching to Place Water Maze, T-Maze, 5 Trial Social Memory, Fear Conditioning**

Objective	**The aim of this study is to determine the behavioral phenotype of the Ts65Dn mouse model of Down syndrome and to establish appropriate behavioral tests for future pharmacological studies.**

Project Title	Behavioral phenotyping of the Ts65Dn mouse model for Down syndrome
Species	Mouse
Strain	Ts65Dn (B6EiC3Sn-a/A-Ts (1716)65Dn)
Sex	Male
Age	9-12 months

Subjects

Group	# of mice	Treatment
Ts65Dn	6-17	none
2N	8-21	none

*Number of mice used varied from test to test

T-Maze

Exploratory alternation behavior was measured in the T-maze. The T-maze had three equal arms (30 cm length, 10 cm width, and 20 cm height); the start arm and two goal arms had guillotine gates as previously described by Belichenko et al. (2009) and Deacon and Rawlins (2006) (Belichenko et al., 2009a; Deacon and Rawlins, 2006). This test is based on the rodents' preference to experience a new arm of the T-maze instead of a familiar one (Gerlai, 1998). In each trial the mouse was placed in the start arm. The gate was opened and the mouse was able to freely explore the arms. As soon as the subject entered one goal arm, the sliding gate of the other goal arm closed. The mouse eventually returned to the start arm and the next trial was started. In the next trial, the mouse may visit the previously chosen goal arm (no alternation) or choose to explore a new arm (alternation). This trial was repeated 11 times per day for 3 consecutive days, for a total of 33 trials. The maze was cleaned with 10% ethanol between trials to eliminate odor traces. Percent of alternation (number of turns in each goal arm) was determined for analysis.

Delayed Matching-To-Place (DMP) Water Maze

The delayed matching-to-place (DMP) water maze task was used to assess learning and memory as originally designed by Steel and Morris (1999) for rats (Steele and Morris, 1999). Subjects were given a series of four trials approximately 8-10 minutes apart in a large water tank (178 cm in diameter) filled with opaque water at a temperature of 22.0 ± 1.5°C. A 15 cm circular platform was submerged 1 cm below the water surface and placed pseudo-randomly in the pool with daily changes in the position. The release point in the pool was changed based on the experimental setup. Each animal was given a maximum of 90 seconds to find the submerged platform. If they were unable to find the platform in that time, the animal was physically guided to it. After remaining on the platform for 10 seconds, the animals were removed and placed in a dry cage. This process was repeated for 7 days. After training in the DMP task, subjects were given visible platform training to ensure they had no gross sensorimotor or visual deficits. During visible platform training, the platform was marked with a black and white ping-pong ball attached to a 10-cm wooden stick. The swim paths of the animals were recorded with the Ethovision 3.1 computer-interfaced camera tracking system (Noldus Information Technology, Wageningen, the Netherlands) and subsequently analyzed. Throughout these tests the water was frequently changed and the tank disinfected.

5 Trial Social Memory Testing

Prior to testing, randomly selected individually housed ovariectomized C57Bl/6J female mice (OEFs) were put into the home cages of subject mice 4 hours per day for 5-7 days to reduce sexual interest. Subject mice were 9 or 18 months old in all the social tests. Trials of all tests were videotaped and subsequently analyzed for olfactory investigation. Investigation was defined as nose-to-body contact of the test animal versus the intruder. Anogenital investigation, perioral investigation, and body investigation were scored in these tests. A single OEF (SAME) was introduced into the home cage of an unfamiliar test animal for four 1-minute exposures with an ITI of 10 minutes for a total of 4 times. In a fifth trial, 10 minutes later, instead of the familiar OEF, a novel, unfamiliar OEF (NOVEL) was put into the home cage of the test animal for one minute.

Fear Conditioning

Contextual and cued fear conditioning was conducted for evaluation of fear-dependent learning and retrieval in the study. The test was performed using chambers from Coulbourn Instruments (Whitehall, PA). On the first day animals were placed in a chamber (Context A) for 3 minutes for baseline recording, followed by five tone-shock pairings. The shock (0.5 mA, 2 sec) was delivered following the tone (70 dB, 2 kHz, 20 sec) in each conditional/unconditional stimulus pairing. On the second day a novel chamber (Context B; new room, new olfactory environment, new texture of floor, blue plastic inserts for walls, extra source of blue light, and visual cues) was used for cued testing. Following a 3-minute pre-tone period, three tones without shocks were presented to animals during a 3-minute testing period. On the last day of the experiment, the mice were placed in Context A for 5 minutes without any conditional or unconditional stimulus {modified from the method described by (Saxe et al., 2006)}. Freezing was defined as the complete lack of motion for a minimum of 0.75 seconds as measured by FreezeFrame software (Actimetrics, Evanston, IL). The percentage of freezing in each period was reported.

T-Maze

% Alternation

2N	Ts65Dn
30	40
80	50
70	30
60	20
80	70
60	40
80	
50	
90	
70	
70	
80	
60	
70	
90	

DMP Water Maze - Escape Latency (s)

Trial	Ts65Dn							
1	80.58143	81.75285	78.88	59.07429	73.64571	78.52143	89.99	86.33
2	76.86285	89.99	68.39857	73.74571	58.17429	89.99	89.99	71.13
3	59.18857	87.96	70.81429	63.70857	89.99	64.12286	89.99	65.88143
4	50.32286	81.23857	60.81857	46.03286	69.32858	89.99	85.78571	80.03714

Trial	2N							
1	39.98429	74.33143	66.43857	65.55143	49.92286	68.84143	73.11714	66.76714
2	55.82857	38.15429	64.82286	58.26	55.37	49.05143	84.94286	41.38714
3	45.56143	31.90571	37.58286	57.30143	65.67	46.83429	61.27714	25.88428
4	40.91429	25.38428	32.97857	48.82143	52.51286	28.14571	54.56857	9.384286

5 Trial Social Memory Test

Trial	Ts65Dn									
1	8.86	9.55	6.61	18.09	3.85	26.28	11.54	14.69	25.24	28.69
2	2.6	3.84	14.67	12.69	9.77	27.13	22.32	15.58	28.16	26.97
3	5.03	1.78	6.43	3.3	13.81	27.06	20.46	19.94	24.81	20.56
4	3.07	6.09	4.86	16.33	3.75	17.41	26.68	13.07	24.72	21.72
Novel	11.29	5.42	5.05	13.78	16.54	23.37	21.37	16.79	27.53	28.72

Trial	2N								
1	23.12	25.28	22.87	19.98	27.38	21.47	28.94	25.69	24.66
2	25.37	23.24	21.53	23.06	23.19	21.58	21.96	27.75	19.79
3	18.11	21.57	12.59	28.06	24.68	20.9	17.38	19.5	10.25
4	13.77	15.33	8.28	26.98	27.29	9.01	26.31	8.8	14
Novel	24.21	26.96	23.88	24.12	28.28	26.94	28.99	29.51	19.03

Cue and Context Fear Conditioning - Percent Freezing

Training

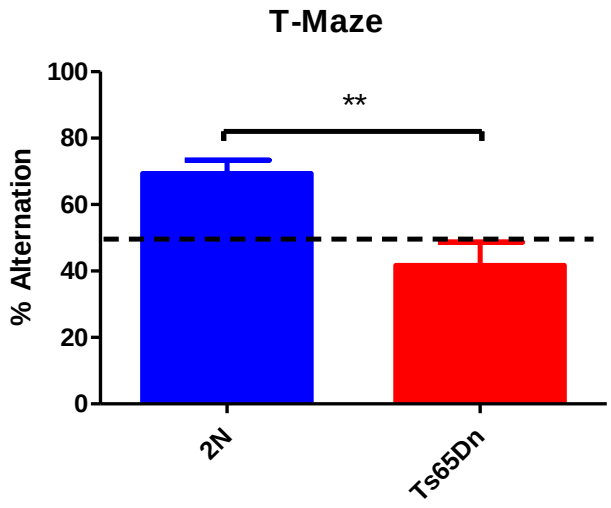
	Ts65Dn																
Baseline	0.4	0	0	3.5	0	1.3	0	0	0	0.4	0	0	0	1.5	0	0.8	2.1
ITI1	28	0	4	45	1.7	6	25	1.3	0	7.7	17	2	6.7	1	37	5	61
ITI2	25	11	0	60	0	15	30	13	2	2.3	55	4	37	18	34	16	58
ITI3	24	8.7	17	78	3.3	7	36	18	1.7	3.4	62	12	56	7.3	51	1.7	86
ITI4	61	16	31	72	6.3	18	71	43	38	2.3	77	24	31	7.4	46	16	80
ITI5	54	1.3	29	66	16	21	64	66	24	38	66	17	12	7.7	50	21	92

Segment	2N																			
Baseline	0	0	0	0	0	0	0	0	0	0	0	0.4	0	0.4	0	0	0	2.27	0	2.27	0.5
ITI1	0	0	35	15.1	1.67	6.01	0	0	0	0	1.69	0	0	8.33	1	5.33	3.33	11.7	1	4.41	1.33
ITI2	0	1.33	39	33.7	3	0	5.67	20.7	0	0	35.7	2.67	8.67	10	4.33	22.3	28	9.67	1	12.4	0
ITI3	9.67	14	49.7	21	28	43.7	25	43.3	42	13.3	73.3	11	38.3	19.5	42.4	46.7	50.7	33.7	1.33	22.7	4.06
ITI4	32.7	21	67.3	41	20.6	57.4	21.3	62.9	48.2	28.4	31.6	22.8	73.9	56	60	22.7	50.7	17.3	0	43.8	6.76
ITI5	64.9	24.9	53	61.5	67.3	78.2	30.4	70.6	52.3	22.8	0	42.5	45.3	39.8	50.1	38.4	74.6	46.4	0	21	0

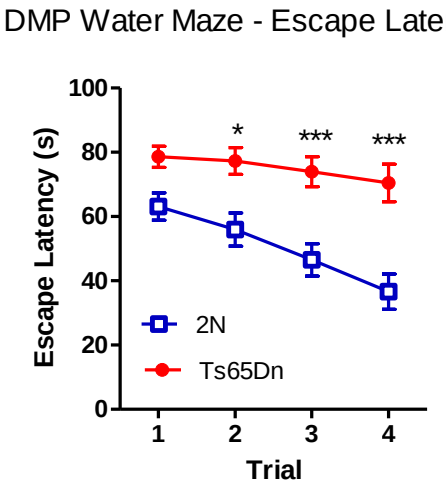
Testing

Test	Ts65Dn																
Cued	25	9.27	7.14	16.3	11.8	21.4	5.08	34.1	29.5	31.6	36.9	23.4	5.66	18.5	30.5	17.5	45.7
Context	42.8	3.04	2.41	2.68	1.96	3.21	1.61	33.4	0.72	1.62	4.28	6.09	3.93	3.04	4.03	13.9	6.07

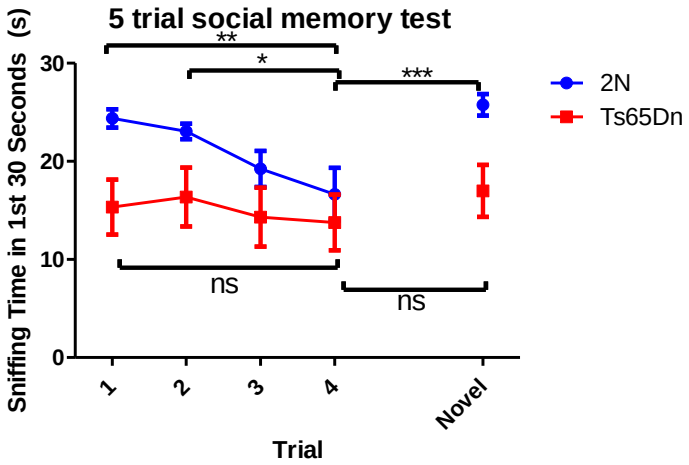
Test	2N																			
Cued	14.6	4.47	49.1	49.6	37.6	36.6	17.2	48.8	7.47	16.3	30	19.3	11.8	57.3	12	47.1	29.2	50.5	51.8	47.6	14
Context	4.39	2.06	40.8	32.6	10.8	28.8	10.6	6.91	10.8	4.07	4.22	9.07	2.23	33.3	8.2	64.4	3.8	60.2	30	33.5	5.08



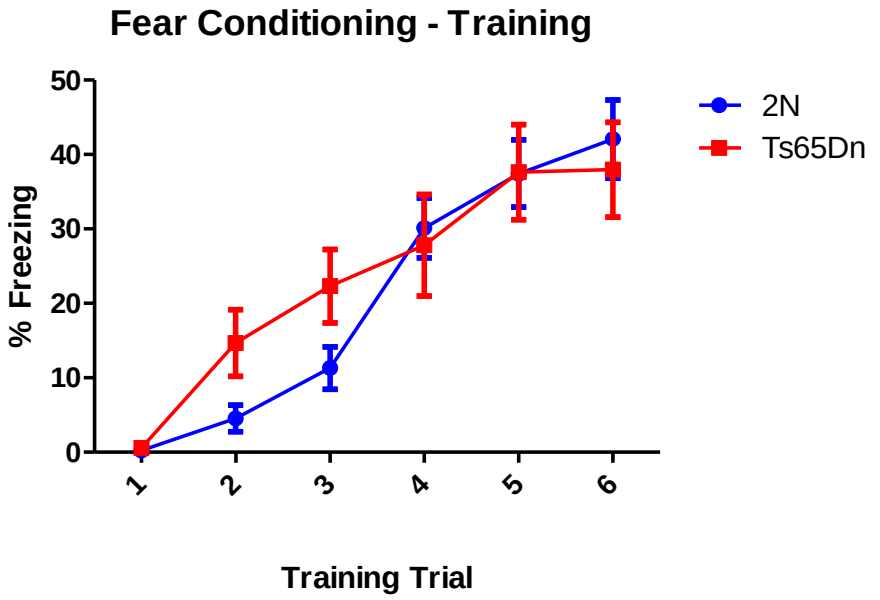
There was a significant difference in percent alternation between 2N and Ts65Dn mice in an unpaired t-test, two-tailed: t(19)=3.539, p=0.0022). Mean and SEM are shown.

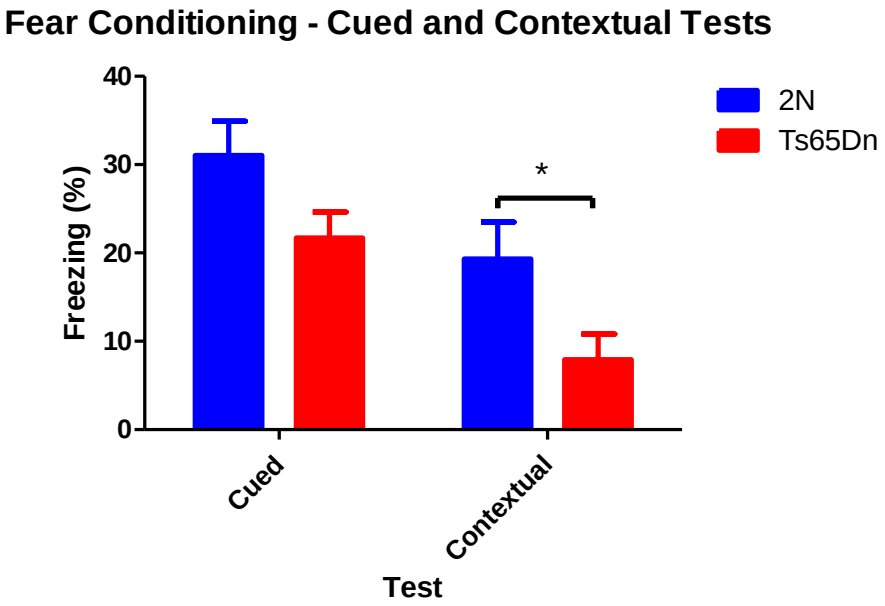


There was a signifcant difference in escape latency between 2N and Ts65Dn mice (2-Way RM ANOVA, Genotype F(1,42)=24.98, P=0.0002)) and Bonferroni post-hoc analysis showed Ts65Dn escape latency was significantly higher in trials 2, 3, and 4 ($P < 0.05$, $P<0.001$, $P<0.001$ respectively)



In the first 30 seconds of the 5-trial social memory test trials, Ts65Dn mice displayed no habituation, whereas 2N mice exhibited a significant habituation to the familiar overectomized female (OEF) and a significant dishabituation to the novel OEF (Two-Way RM ANOVA Gentoype: $F(1,17)=4.94$, $P=0.0402$, followed by Bonferroni post-hoc analysis).





2N and Ts65Dn mice aquired the task similarily during training (Top, Two-Way RM ANOVA Genotype: F(1,36)=0.29, P=0.5938). Ts65Dn mice showed a significant deficit in the contextual memory task (Unpaired t-test, two-tailed t(36)=2.149, P=0.0384), but not in the cued memory task (Unpaired t-test, two-tailed t(36)=1.866 P=0.0703).

In conclusion, we show that the Ts65Dn mouse model of Down Syndrome has a robust behavioral deficit and is thus a good experimental model for drug discovery studies. Ts65Dn mice demonstrated significantly fewer alternations than 2N mice in T-maze, higher escape latentcies in DMP water maze, and deficits in contextual memory during fear conditioning. They also showed a lack of habituation to a familiar OEF and a lack of dishabituation to a novel OEF during 5 trial social memory.