Published in final edited form as:

Behav Brain Res. 2009 October 12; 203(1): 137–142. doi:10.1016/j.bbr.2009.04.031.

Does the learning deficit observed under an Incremental Repeated Acquisition schedule of reinforcement in Ts65Dn mice, a model for Down syndrome, change as they age?

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Abstract

The Ts65Dn mouse is partly trisomic at chromosome 16 and is considered to be a valid mouse model of human Down syndrome. Prior research using an incremental repeated acquisition (IRA) schedule of reinforcement has revealed that there is a significant learning deficit in young, adult Ts65Dn mice compared to littermate controls. The purpose of this study was to examine whether this deficit changes during the life-span of these mice. In order to determine if changes in the deficit were caused by motoric or motivational deficiencies, a second group of mice was trained to respond under a performance version of the task (IRA-P). The IRA-P task required the same motor responses to produce the reinforcer, but no learning or acquisition was required. Data collected under the IRA task demonstrated that there was a significant learning impairment that persisted up to 24-months of age in the Ts65Dn mice compared to littermate controls. There was a significant decrease in the rate of responding and the number of milk presentations earned by the Ts65Dn mice after 19-months of age. However, during this time, response accuracy, which is independent of mobility and possibly motivation, did not decrease. Under the IRA-P schedule, there was no decrease observed in the number of milk presentations of either line as they aged, but the trend in the rate of responding of the Ts65Dn mice was similarly declining as the rate of responding observed in the Ts65Dn mice under the IRA task. These data indicate that the ability to learn in Ts65Dn mice does not decline with age as measured by the IRA task and suggests that perhaps Ts65Dn mice do not exhibit the same early-onset Alzheimer's disease phenotype that is typically seen in human patients.

Introduction

Down syndrome is the most frequently occurring cause of mental retardation of genetic origin. It is caused by a trisomy of chromosome 21. Down syndrome patients typically have a learning deficit associated with mental retardation. In addition, a large percentage of Down syndrome patients develop an early onset form of Alzheimer's disease [16]. Mouse models of Down syndrome possess syntenies, or chromosomal regions in which homologues are arranged in the same order, in both humans (chromosome 21 (HSA21)) and mice (chromosome 16 (MMU16)) [11] as well as on HSA21 and MMU10 and also on HSA21 and MMU17 [8]. Approximately 80% of MMU16 and HSA21 are syntenic [11]. This homology is one of the

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benefits of using mouse models of Down syndrome. One such model, the Ts65Dn, has a partial trisomy of MMU16 and survives postnatally [5]. The 17¹⁶ translocation chromosome has the murine orthologues of genes from the Down syndrome-critical region, which is believed to cause many of the neurological deficits seen in Down syndrome [1,10]. The Ts65Dn mouse demonstrates many of the same problems that are seen in Down syndrome patients, such as craniofacial maldevelopment [21], hyperactivity, and neurochemical disparities [13]. Hunter *et al.*, have shown that there is a decline in the number of cholinergic neurons in the basal forebrain in the Ts65Dn mice [13]. The Ts65Dn mice have also been demonstrated to have abnormal synaptic elements in the hippocampus compared to their littermate control mice [4]. The cognitive abilities of these mice are still unclear. While there have been studies that have examined learning in Ts65Dn mice at different ages, there has been no study that has examined learning continuously over a significant portion of the life-span. The study presented here will attempt to determine if Ts65Dn mice display an accelerated rate of decline in their learning ability indicative of the cognitive decline observed with the early onset Alzheimer's disease that is observed in human patients with Down syndrome [16].

Hyde and colleagues [14] reported a learning deficit in the Ts65Dn mice while performing a contextual discrimination task, which makes use of foot shock, and several investigators have demonstrated that Ts65Dn mice exhibit increased search times in water mazes [20,9,12,18, 22]. However, foot shock and water mazes may cause stress that can affect the animals' performance. Multiple studies have demonstrated that stress can affect cognitive function in laboratory animals [6,7,17,25]. Water mazes also rely heavily on the motor function of the laboratory animal and Costa *et al.* [4] has demonstrated that Ts65Dn mice have a motor deficit. An alternate learning procedure that does not use any kind of aversive stimuli and does not require perfect motoric ability is the incremental repeated acquisition (IRA) procedure [24], a modified repeated acquisition procedure [19]. A second, and important, advantage of the repeated acquisition procedure is that allows for the daily examination of learning over the lifespan of the mouse.

IRA procedures allow for task difficulty to be varied from easy to difficult during a single test session. A similar type of procedure was first used by Weinberger and Killam [23] in order to measure the effects of chronic diazepam and phenobarbital treatment on learning at different levels of task difficulty in baboons. During IRA sessions, subjects learn a chain, or sequence, of responses that results in the presentation of a reinforcer. At the beginning of each session only a single response is required to produce the reinforcer. As the session progresses, additional responses are required and the ultimate response chain length is determined by the investigator and/or the ability of the subject.

In 2004, Wenger *et al.*, used an IRA schedule with four different response chain lengths to examine learning in young adult Ts65Dn males compared to young adult littermate control males [24]. During this procedure the mice were required to produce a 1-response chain for each of the first 5 milk presentations, a 2-response chain for each of the next 10 milk presentations, a 3-response chain for each of the following 30 milk presentations, and a 4-response chain for each of the final 20 milk presentations. Using this procedure Wenger and colleagues found that Ts65Dn and littermate control mice could both be trained to perform an IRA task. However, Ts65Dn mice typically did not earn all of the milk presentations available when the 3-response chain was required, while littermate control mice were frequently able to earn all of the milk presentations that required a 3-response chain and several littermate controls could earn many of the milk presentations that required a 4-response chain. These data suggested that task difficulty was an important factor when investigating learning in Ts65Dn mice [24]. The IRA task for mice, as described by Wenger *et al.* [24] was chosen for use in the present studies. A second task, a performance version of the Incremental Repeated Acquisition (IRA-P) task was used to determine if there was a difference in motivation or the motoric ability

of the mice to perform the task as they age. The IRA-P task required the same motoric responses but differed from the IRA task in that it did not require the acquisition of a new response sequence each day. Furthermore, at each step in the chain the correct response position was illuminated.

The hypothesis for this study was that the Ts65Dn mice have learning deficits compared to littermate control mice and these deficits will change as the mice age. To determine how the learning deficit changed with age both lines of mice began performing under an IRA or IRA-P schedule of food reinforcement at 3-months of age and continued performing under this same schedule until they were sacrificed at 24-months of age.

Materials and Methods

Testing took place in six model ENV-307A mouse operant conditioning chambers (Med Assoc., St. Albans, VT). Each chamber was housed in a model ENV-021M sound-and light-attenuating enclosure (Med Assoc., St. Albans, VT). All chambers had a dipper hole placed flush with the chamber floor in the middle of the front panel of the chamber. A dipper hole provided access to a 0.01 ml dipper of evaporated milk. To each side of the dipper hole there was a nose-poke response module. Each nose-poke response module was equally spaced from the dipper hole and the nearest side wall of the test chamber. A third nose-poke response module was placed opposite of the dipper hole on the back wall. Each of these also had an infrared beam (IR) horizontally crossing the hole and breaking the IR beam registered as a response and produced an audible click. All nose-poke response modules and the dipper hole could be illuminated by an LED. All the chambers were illuminated with a 28V DC bulb. A model SC628H Sonalert in series with a 100 Ω resistor was mounted in the enclosure to provide a tone, in this experiment, when incorrect responses were made.

The experiment began with 24 Ts65Dn and 24 littermate control male mice. The mice were obtained from Muriel Davidson's laboratory at Jackson Labs. All of the mice that were used in these experiments were genotyped and screened for retinal degeneration before being shipped. Each mouse line was divided into two groups. Group 1 (12 Ts65Dn and 12 littermate control mice) started training at approximately 3-months of age on the IRA schedule of reinforcement. Group 2 (12 Ts65Dn and 12 littermate control mice) started training at 3-months of age on IRA-P schedule of reinforcement. The mice were individually housed and maintained on a 12 hour light/dark cycle. Before training began each mouse was food restricted to 85% of his *ad libitum* weight and maintained at that degree of food deprivation throughout the duration of the experiment by limiting post-session food intake. All the mice received *ad libitum* access to water in their home cages. Test sessions were conducted between 8:00 AM and 6:00 PM five days a week, Monday through Friday. All mice were sacrificed at approximately 24-months of age or if they became moribund.

All mice were trained to respond by interrupting the IR beam using an autoshaping procedure similar to that described in Wenger *et al.* (2004) [24]. Training under the IRA schedule of reinforcement began on the tenth training session. Under the IRA schedule mice were required to learn one of six 4-response chains, or sequences, daily. The response chains were made up of responses on the right (R), left (L), or back (B) nose-poke response modules. The six sequences used for this experiment were chosen based on the following criteria: no single response position would be repeated during the chain with the exception that the fourth response of the chain will be the same position as the second response. The six sequences were as follows: Sequence 1, or S1 (R, L, R, B); S2 (L, B, L, R); S3 (B, R, B, L); S4 (L, R, L, B); S5 (B, L, B, R); and S6 (R, B, R, L). Starting on the first day of training, Sequence 1 (S1) was used, the next day S2 was used, and so on. Once all six sequences had been presented, S1 was presented again and the cycle of 6 sequences was repeated. By presenting the sequences in this

order, the response position that produced access to evaporated milk one day would not produce evaporated milk again for two more daily sessions. The response chain was incremented so that for the first 5 milk presentations the mouse was required to produce a 1-response chain. For example if a mouse was responding under sequence 3 (B, R, B, L), the dipper of milk would be presented following each response the mouse made on the left nose-poke response module (L). For each of the next 10 milk presentations the mouse would be required to produce a 2-response chain. Thus, the dipper of milk was presented each time the mouse responded once on the back nose-poke module followed by one response on the left nose-poke module (B, L). Note that for all sequences the new response position is added to the beginning of the chain. Thus, a response on the nose-poke module at the position required for the one response chain remains the response position that immediately precedes milk presentation for all chain lengths in a test session. Each of the next 30 milk presentations required the emission of a 3-response chain, in this case (R, B, L). For the final 20 milk presentations a 4-response chain was required, which would be (B, R, B, L). Any incorrect response resulted in a 3 s time-out. During the timeout all lights in the chamber were extinguished and the Sonalert emitted a 3-s tone. Following the time-out the sequence was re-initiated. So if a mouse was required to respond (L, B, L, R), but it responded (L, B, R), the 3 s time-out began immediately upon the incorrect response, and after the time-out the mouse was required to begin the sequence anew, error correction was not permitted. The session ended after 3600 s or once all 65 milk presentations were earned, whichever occurred first. All three nose-poke response modules were active, indicated by the LED being illuminated, during the entire session, except during the 3 s time-out and during the 5 s access to milk. The methods used in this experiment to train response acquisition and IRA responding are more thoroughly described in Wenger *et al.* [24].

Training under the IRA-P schedule of reinforcement began on the 18th training session. The autoshaping procedure was also similar to the one used in the IRA group with the exception that, due to technician error, the IRA-P group ran under the autoshaping procedure for an extra 8 days. However, because of the similarity of the autoshaping procedure to the actual IRA-P schedule of reinforcement (i.e. only one response position illuminated at a time) we do not believe this error significantly altered the performance of the mice being training on the IRA-P schedule of reinforcement. The IRA-P schedule of reinforcement training was conducted in the same manner as training for the IRA schedule of reinforcement, with the exception that under the IRA-P schedule only the correct response position was illuminated by a stimulus light, and the same sequence of responses was required each day (R, L, R, B).

Analysis of Data

In order to determine how learning performance changed over the life-span of the mice several IRA endpoints were examined. These endpoints included the number of milk presentations earned during each session; the overall session percent accuracy, or percentage of correct responses; and the rate of responding, (the number of responses divided by the total stimulus presentation time). Because of the large amount of data generated over the entire life-span of the mice, the data were analyzed first by averaging individual mouse data collected on Thursdays at each month of age and then finding the average for that month of age for each mouse line. In addition, since a number of drugs were administered acutely over the life-span of these mice, utilizing the data collected on Thursdays of each week minimized any after effects of any of the drugs since they were given only on Tuesdays and Fridays. The data were analyzed using a repeated measures generalized linear model with a Bonferroni correction, and significance was determined at the $p < 0.005$ level.

Results

IRA Task

Figure 1 shows the mean milk presentations earned by each mouse line at each month of age. An analysis of variance shows that the effect of both age ($p < 0.0001$, $F = 20.979$, $d.f. = 20$, $d.d.f. = 373$) and line ($p < 0.0001$, $F = 205.427$, $d.f. = 1$, $d.d.f. = 373$) are significant and there is a significant interaction between age and line ($p < 0.0001$, $F = 5.958$, $d.f. = 19$, $d.d.f. = 373$). There was a significant difference in the number of milk presentations earned by the Ts65Dn mice compared to littermate controls at 7-, 9-, 12-, and 16- through 23-months of age. As the mice aged, a number of changes were observed in their performance. As shown in Figure 1, top panel, the number of milk presentations earned by the Ts65Dn mice started to decrease at approximately 17-months of age. The number of milk presentations earned by the littermate control mice did not decrease with age. Interestingly, while the control mice responded with a higher percent accuracy for the entire session than the Ts65Dn mice, the percent accuracy did not appear to decrease for either mouse line across the life-span as shown in Figure 1, middle panel. An analysis of variance shows that the effect of both age ($p < 0.0001$, $F = 35.475$, $d.f. = 20$, $d.d.f. = 373$) and line ($p < 0.0001$, $F = 224.655$, $d.f. = 1$, $d.d.f. = 373$) are significant and that there is a significant interaction between age and line ($p < 0.0001$, $F = 3.227$, $n.d.f. = 19$, $d.d.f. = 373$). There was a significant difference in the overall session percent accuracy of the Ts65Dn mice compared to littermate controls at 3-, 5- through 10-, 12-, 13-, and 16- through 23-months of age. Figure 1, bottom panel, shows that the reason for the age-related decline in the number of milk presentations earned per session is that there is a dramatic decrease in the rate of responding for the Ts65Dn mice after approximately 16-months of age. A generalized linear model analysis of variance shows that the effect of both age ($p < 0.0001$, $F = 3.181$, $n.d.f. = 20$, $d.d.f. = 373$) and line ($p < 0.0001$, $F = 77.120$, $n.d.f. = 1$, $d.d.f. = 373$) are significant and that there is a significant interaction between age and line ($p < 0.0001$, $F = 3.082$, $n.d.f. = 19$, $d.d.f. = 373$). There was a significant difference in the overall session rate of responding in the Ts65Dn mice compared to littermate controls at 20- through 23-months of age. A similar decrease in rate of responding did not occur in the littermate control mouse line.

IRA-P Task

Figure 2, top panel, shows the mean milk presentations earned by each mouse line at each month of age. A generalized linear model analysis of variance shows that the effect of age were significant ($p < 0.0001$, $F = 7.785$, $n.d.f. = 17$, $d.d.f. = 359$) and there was not a significant interaction between age and line. Figure 2, middle panel, shows the percent accuracy for the entire session. A generalized linear model analysis of variance determined that the effects of age were significant ($p < 0.0001$, $F = 24.954$, $n.d.f. = 20$, $d.d.f. = 359$) and there was not a significant interaction between age and line. Figure 2, bottom panel, shows the rate of responding and a generalized linear model analysis of variance shows that only the effect of line is significant ($p < 0.0001$, $F = 41.893$, $n.d.f. = 1$, $d.d.f. = 359$) and there was not a significant interaction between age and line. However, there was a declining trend in the rate of responding of the Ts65Dn after approximately 17-months of age. It is important to note that while there was a break between 15- and 17-months, due to factors out of our control, accuracy did not decrease following the break. Thus, we do not believe that the decrease in the number of milk presentations and the rate of responding are the result of the break. It is far more likely that these decreases beginning at 17 months of age are due to the same age-related decreases in motor function, and possibly motivational as observed in the mice responding under the IRA schedule.

As mentioned previously mice were sacrificed at 24-months of age or if they became moribund. Figure 3 shows survival curves for each mouse line in each group. The Ts65Dn clearly have

a lower survival rate than the littermate control mice, and the results here are similar to that reported by Muriel Davisson (personal communication).

Discussion

It seems clear that there is a learning deficit over the entire life-span of the Ts65Dn mice compared to their littermate controls. While there were some months of age where the mean number of milk presentations earned during the IRA task was not statistically significant between the Ts65Dn mice and their littermate controls (particularly when they were younger), the number of milk presentations earned by the Ts65Dn mice is always less than that for their littermate controls. This is not the case during the IRA-P task. Prior to 17-months of age, both lines of mice responding under the IRA-P task earned approximately the same number of milk presentations. This strongly suggests that the difference in the number of milk presentations over the first 17-months or so of the life-span of the Ts65Dn mice responding under the IRA task is due to a decreased acquisition, or learning, compared to the littermate controls and is not the result of a difference in motor function or motivation to respond. While the role of the hippocampus in the IRA task has yet to be studied, a non-operant version of this task has been shown to be dependent on normal functioning of the hippocampus [3]. Thus, considering the decreased synaptic plasticity in the hippocampus of Ts65Dn mice [2,15] our results are not necessarily surprising.

As the Ts65Dn mice aged there was a declining trend in the number of milk presentations earned under both the IRA (learning) and the IRA-P (non-learning) task. This suggests that the decrease observed under the IRA task may not be the result of a decreased learning ability in the aged Ts65Dn mice. Rather the decline in the number of milk presentations earned beginning at about 17-months of age under both the IRA and IRA-P schedules suggests that declining motor function and/or motivation may be the most likely explanation. It is important to note that although rate of responding is declining in the Ts65Dn mice under both the IRA and IRA-P tasks, accuracy is not declining under either task during these last months of the experiment. If accuracy, under either task, also declined during these latter months, it could be argued that acquisition or learning also was declining, however, in the absence of a decline in accuracy there is little evidence in these data to support an age-related decrease in learning in the Ts65Dn mice.

Interestingly, the apparent greater decline in motor function and/or motivation in the Ts65Dn mice parallels the accelerated decline observed in the survival curves for the Ts65Dn mice compared to the littermate controls. In addition, although it was not objectively measured, laboratory personnel reported that the Ts65Dn mice moved about in their home cages with great difficulty, compared to littermate controls, after about 19 months of age. These data show that while a learning deficit in the Ts65Dn mice compared to littermate controls remains at 24-months of age, a decline in motor function and/or motivation would appear to account for the decrease in milk presentations earned during the last months of the study. Thus, this data does not support the hypothesis that the learning deficit changes with age in the Ts65Dn compared to littermate controls.

There are several important issues to consider when asking why these data do not support the hypothesis. The first possibility is that since the mice were tested daily from 3- to 24-months of age, the daily testing may have masked any age-related decline in learning. This is hard to rule out in a study using such a long-term daily testing paradigm. A second possibility is that the Ts65Dn mice may not possess the premature form of Alzheimer's disease phenotype observed in a high percentage of Down syndrome patients [16]. This, likewise, is difficult to prove since it essentially would require proving a null hypothesis. The fact that we were unable to show an age-related decrease in learning is also interesting especially in light of the

documented loss of basal forebrain cholinergic neurons [13]. All that can be said is that using this procedure we did not find support for the hypothesis that Ts65Dn mice show an age-related decline in learning function analogous to the development of Alzheimer's disease in human Down syndrome patients.

Finally, it is important to note that the research presented was part of a larger study that also examined the effects of cognitive enhancers on learning in the Ts65Dn mice, and this may have influenced our results. These purported cognitive enhancers were as follows: nicotine bitartrate, a nicotinic agonist (0.03–3.0 mg/kg) at 6-months of age; *d*-amphetamine sulfate, a stimulant, (0.1–10 mg/kg) at 9-months of age; rolipram, a phosphodiesterase inhibitor, (0.03–1.0 mg/kg) at 11-months of age; memantine hydrochloride, an NMDA receptor antagonist, (0.1–18 mg/kg) at 13-months of age; tacrine hydrochloride, an acetylcholinesterase inhibitor, (0.03–5.6 mg/kg) at 15-months of age; ABT-418, also a nicotinic agonist, (0.03–3.0 mg/kg) at 18-months of age; *d*-amphetamine sulfate (0.1–10 mg/kg) at 20-months of age; rolipram (0.03–1.0 mg/kg) at 21-months of age; memantine hydrochloride (0.3–30 mg/kg) at 22-months of age; and tacrine hydrochloride (0.3–5.6 mg/kg) at 23-months of age. During the determination of the dose-response curves for each of these agents, doses were administered on Tuesday and Friday of each week with saline control injections conducted on Thursdays. It should be noted that the data presented in this study is from the Thursday session of each week. Thus, it is unlikely that any acute effects of these drugs are reflected in the data presented. Nevertheless, it is interesting to note that the peak in the number of milk presentations earned on Thursdays of each week by the Ts65Dn mice occurred at approximately 12 months of age, the time during which a dose-response curve for rolipram was being conducted. However, it should be noted that rolipram had little effect on the number of milk presentations earned on Tuesdays and Fridays (the days on which it was administered prior to the daily test session). These data will be submitted for publication shortly.

Acknowledgments

This work was supported in part by the NIEHS pre-doctoral training grant 5T32ES01952-04 and NIH grant HD047656 (GR Wenger). A special thank you to Camron Hall for providing lab support.

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Figure 1.

Top Panel: Average IRA number of milk presentations earned during the session for the Thursdays of each month of age. X-axis: month of age; y-axis: milk presentations. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice from which data were collected during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursday of data collected. A § indicates $p < 0.005$.

Middle Panel: Average IRA session percent accuracy for the Thursdays of each month of age. X-axis: month of age; y-axis: session percent accuracy. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice from which data were collected for

during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursday of data collected. A § indicates $p < 0.005$.

Bottom Panel: Average IRA session rate of responding for the Thursdays of each month of age. X-axis: month of age; y-axis: responses per second. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice data was collected for during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursday of data collected. A § indicates $p < 0.005$.

Figure 2.

Top Panel: Average IRA-P number of milk presentations earned during session on Thursdays of each month of age. X-axis: month of age; y-axis: milk presentations. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice from which data were collected during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursdays of data collected.

Middle Panel: Average IRA-P session percent accuracy for the Thursdays of each month of age. X-axis: month of age; y-axis: session percent accuracy. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice from which data were collected.

for during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursday of data collected.

Bottom Panel: Average IRA-P session rate of responding for the Thursdays of each month of age. X-axis: month of age; y-axis: responses per second. Each data point represents the mean $\pm$ S.E. of each mouse line; (n) represents the number mice data was collected for during that month of age. An * indicates months where fewer than four Thursdays of data were collected. ** indicate months where there were five Thursday of data collected.

Survival Curves for Ts65Dn mice and Littermate Controls

Figure 3.

Number of Mice to survive to each month of age. X-axis: month of age; y-axis: number of mice alive.