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Research report

Working memory in the aged Ts65Dn mouse, a model for Down syndrome

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ABSTRACT

The Ts65Dn mouse displays several phenotypic abnormalities that parallel characteristics found in Down syndrome. One important characteristic associated with Down syndrome is an increased incidence of early-onset Alzheimer's disease. Since Alzheimer's disease is characterized largely by progressive memory loss, it is of interest to study working memory in the Ts65Dn mouse. Previous research in our lab using a titrating, delayed matching-to-position schedule of reinforcement has demonstrated that young, adult male Ts65Dn mice do not display a working memory deficit when compared to age-matched littermate controls. However, there have been no studies examining the working memory of these mice as they age. Due to the correlation between Down syndrome and Alzheimer's disease, and as part of a larger effort to further characterize the phenotype of the Ts65Dn mouse, the purpose of this study was to determine whether aged Ts65Dn mice possess a working memory deficit when compared to age-matched littermate controls.

In order to study working memory, two groups of mice were trained under a titrating, delayed matching-to-position schedule of reinforcement. The first group was trained beginning at 3 months of age, and the second group began training at 15 months of age. Both groups were studied to 24 months of age. Initially, both groups of Ts65Dn mice performed at a lower level of accuracy than the control mice; however, this difference disappeared with further practice. The results from these lifespan studies indicate that the aged Ts65Dn mouse does not possess a working memory deficit when compared to age-matched controls.

1. Introduction

Down syndrome, a condition resulting from the presence of three copies of chromosome 21, is the most common genetic cause of mental retardation, occurring in approximately 1 out of every 700 live births [4]. Individuals with Down syndrome possess a range of phenotypic characteristics, including craniofacial abnormalities, muscle hypotonia, and an increased susceptibility to infection. In addition, perhaps the most striking consequence of an extra copy of chromosome 21 is the mental retardation that, although varying in degree, is universally present in individuals with Down syndrome. This mental retardation is manifested clinically by impaired learning and memory [see reviews: 29,41]. Patients with Down syndrome also display an increased incidence of early-onset Alzheimer's disease, with almost all patients over the age of 35 displaying characteristic Alzheimer-like neuropathology [see reviews: 2,41].

Experimental animal models are a useful resource for studying many human conditions. Mouse models in particular offer a unique perspective due to the many metabolic and anatomical similarities between mouse and human. The relatively short lifespan and short generation time of the mouse also make these animals an excellent choice in the laboratory. Perhaps more importantly for studying human aneuploid conditions, however, is the considerable genetic similarity between the two species. Specifically, substantial homology exists between human chromosome 21 (HSA21) and mouse chromosomes 10, 16, and 17, with the majority of the conserved segments found on mouse chromosome 16 (MMU16) [21,38]. The Ts65Dn mouse, developed by Muriel Davisson at The Jackson Laboratory, has a partial trisomy of MMU16. In these mice, 80% of the genes conserved between MMU16 and HSA21 are found in triplicate. Because of this high degree of homology, the Ts65Dn is generally considered the best animal model of Down syndrome [1,10]. In addition, these mice display several phenotypic abnormalities which parallel those found in humans with Down syndrome. These abnormalities include developmental delays, hyperactivity [11], craniofacial malformations [33], motor dysfunction [6], and learning deficits [18,35,40].

Working memory can be defined as a temporary system for maintaining and manipulating information; more simply, it is the memory required to perform accurately on a specific task. Some studies have reported working memory deficits in individuals with

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Down syndrome [25,30]. Thus, in an effort to further characterize the phenotype of the Ts65Dn mouse, it is of interest to determine whether these mice also possess working memory deficits. Several methods have been developed to study working memory in laboratory animals. One of the most widely used methods is the Morris water maze, in which the subjects are placed in a tank of water and required to remember the location of a platform hidden under the surface of the water. Studies in the Ts65Dn mouse using the Morris water maze have suggested that these mice do indeed possess working memory deficits [19,22]. However, the Morris water maze may not be the most appropriate method for studying memory, particularly in the Ts65Dn mouse. Not only can the water maze produce increased levels of stress, which in turn may adversely affect performance, but Ts65Dn mice have demonstrated both slower swimming speeds and significant thigmotaxis (swimming around the perimeter of the water tank), which may make interpretation of results difficult [6,18].

Behavior can be studied using operant conditioning procedures, which are based on the principle that responses (behavior) are controlled by their consequences. Although it can take a considerable amount of time to train animals under operant procedures, operant conditioning provides several advantages over other methods. Not only can animals be studied without the use of aversive stimuli, but testing can be performed on the same animals for extended periods of time (months or even years). The schedule of reinforcement used to study working memory in these experiments is a titrating, delayed matching-to-position schedule, a modification of the delayed matching-to-sample method developed for use in pigeons [3]. Under this schedule, the animal is required to remember a response position during a delay period and respond at that same position ('match') following the delay. A previous study in our lab using this schedule demonstrated that young (3–6 months old) Ts65Dn mice do not show a working memory deficit when compared to littermate controls. Though initially there appeared to be a deficit in the Ts65Dn mice, the discrepancy in performance between the two groups disappeared with further training [17].

The current study represents the first longitudinal study of working memory in the Ts65Dn mouse, which is important for a number of reasons. First, little is known about the cognitive abilities of these mice as they age, and in humans, memory loss is a frequently reported side effect of age. Second, there is a decided link between Down syndrome and Alzheimer's disease. Multiple studies have demonstrated that virtually every person with Down syndrome will eventually display the characteristic neuropathology of Alzheimer's disease [9,24,42,43, see review: 26]. Though the Ts65Dn mouse does not exhibit amyloid plaques or neurofibrillary tangles, they do show an age-related degradation of basal forebrain cholinergic neurons (BFCN), similar to that seen in Alzheimer's disease [5,20,27,36]. This leads to our hypothesis that an accelerated decline in working memory, compared to their euploid littermates, will be observed in Ts65Dn mice as they age. Thus, the purpose of the studies reported here was to determine whether an age-related deficit in working memory is present in the Ts65Dn mouse.

2. Materials and methods

2.1. Animals

Twenty-five male Ts65Dn mice and 25 male littermate control (LC) mice were obtained from The Jackson Laboratories (Bar Harbor, ME). All mice were genotyped and screened for retinal degeneration before being shipped. Beginning at approximately 3 months of age, animals were housed individually and maintained on a 12-h light/dark cycle. The animals were given water ad libitum and food deprived to 85% of their free-feeding weights.

2.2. Apparatus

Subjects were tested in four Med Associates (St. Albans, VT) Modular Mouse Test Chambers (ENV-307A), which were housed in sound- and light-attenuating enclosures (Med Assoc., St. Albans, VT). On the front wall of each chamber there was a center dipper hole, which provided access to a 0.01 ml dipper of evaporated milk. Also on the front wall were two nose-poke holes (NPH), equally spaced on either side of the center dipper hole. There was a third NPH on the back wall of the chamber, directly opposite the front dipper hole. Each NPH (including the dipper hole) could be illuminated by an LED, and each had a beam of infrared light crossing horizontally in front of the LED. A response was recorded when a mouse inserted its nose into the NPH, interrupting the infrared beam. An audible click sounded with each response as a means of feedback. Each chamber was illuminated by a 28 V DC bulb, and equipped with a model SC628H Sonalert in series with a 100 Ω resistor that sounded a tone when incorrect responses were made.

2.3. Behavioral testing

The mice were separated into two groups: Group 1 consisted of 24 mice (12 Ts65Dn and 12 LC), while Group 2 consisted of 26 mice (13 Ts65Dn and 13 LC). At approximately 4 months of age, the mice in Group 1 began response acquisition training. The mice in Group 2 were kept under the same environmental conditions as the mice in Group 1 (food deprivation, daily handling, etc.), but did not begin response acquisition training until 15 months of age. The behavioral testing was conducted five days a week, Monday through Friday, from approximately 8:00 a.m. to 5:00 p.m. All mice were sacrificed after the conclusion of the study or if they became moribund. Data for those mice that expired or were sacrificed prior to the end of the study were included up until the point of death; in cases where the behavior declined precipitously prior to death, data were included up until the point of decline.

Initially, the animals underwent response acquisition training, in which they learned to respond in the test chamber. Under response acquisition training, each training session lasted for 30 min or until 50 reinforcers were delivered. During the first session of response acquisition training, an alternative fixed-ratio 1, fixed-time 60 s (alt FR1 FT60) schedule of reinforcement was utilized. Under this schedule, the mouse was reinforced (presented with a dipperful of evaporated milk) after each response on the dipper hole or after a 60-s period of no responding. During the second session, the schedule was changed to fixed-ratio 1 (FR1), such that the animals were reinforced for each response on the dipper hole. In the third and fourth sessions, the animals were reinforced only for responding on the left front NPH; in the third session, the animals were reinforced for every response (FR1) on the left NPH, while in the fourth session, they were reinforced for every 3rd response (FR3) on the left NPH. In the fifth and sixth sessions, the animals were reinforced for completing a FR3 on the right NPH. During the seventh and eighth sessions, the animals were reinforced for responding on the back NPH; in session seven, they were reinforced for every completion of a FR3, while in the eighth session, the fixed-ratio requirement was increased to 10 (FR10). For the next two sessions, the schedule of reinforcement was a FR10 random; i.e. within each session, the animals were reinforced for completing a FR10 on a randomly selected NPH (left, right, or back). Response acquisition training was considered complete when the animals could earn at least 10 reinforcers per session on two consecutive sessions on the FR10 random schedule.

Once the animals had completed response acquisition training, they were trained under a matching-to-position (MTP) schedule of reinforcement as previously described [17]. Under this schedule, each session lasted for 60 min or 50 trials, whichever came first. At the start of each trial, either the left or the right (randomly selected) front NPH was illuminated and the animal had to complete a fixed-ratio 5 (FR5) at that NPH. Upon completion of the FR5, the LED illuminating that NPH was extinguished, the back NPH was illuminated, and a delay period began. The first response at the back NPH after the delay period had elapsed extinguished the LED illuminating the back NPH and illuminated both left and right front NPH. If the animal responded at the same front NPH as before the delay (a 'match'), the animal was reinforced with 5-s access to the dipper of evaporated milk. If the animal responded at the opposite front NPH (a 'non-match'), a tone sounded, initiating a 5-s time-out period during which all lights in the chamber were extinguished. Following the time-out period the animal had to repeat that trial.

Initially, the mice were trained using a fixed 3-s delay period. However, using a fixed delay can be problematic. If a delay is selected that is too short, the animals will encounter a 'ceiling effect', under which they get 95–100% correct. At such a high level of accuracy, it is difficult to demonstrate improvement. If a delay is selected that is too long, accuracy will approach 50%, which in a 2-choice procedure is difficult to distinguish from chance. Hence, a titrating delay was implemented. Under the titrating schedule, the delay remained fixed at 3 s for the first five trials of each session. On the sixth and all subsequent trials, the length of the delay was determined by the performance on the preceding five trials. If the animal made the correct choice ('match') on 5 out of the 5 previous trials, the delay increased by 3 s for the next trial. If the animal made the correct choice on 4 out of the 5 previous trials, the delay remained the same for the next trial. If the animal made the correct choice on 3 or less out of the previous 5 trials, the delay decreased by 3 s for the next trial, with a minimum delay of 3 s. This procedure kept each animal performing at approximately 80% accuracy, which has two advantages: first, the task difficulty is standardized across subjects, and