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ORIGINAL ARTICLE

Attentional function and basal forebrain cholinergic neuron morphology during aging in the Ts65Dn mouse model of Down syndrome

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Abstract Individuals with Down syndrome (DS) exhibit intellectual disability and develop Alzheimer's disease-like neuropathology during the third decade of life. The Ts65Dn mouse model of DS exhibits key features of both disorders, including impairments in learning, attention and memory, as well as atrophy of basal forebrain cholinergic neurons (BFCNs). The present study evaluated attentional function in relation to BFCN morphology in young (3 months) and middle-aged (12 months) Ts65Dn mice and disomic (2N) controls. Ts65Dn mice exhibited attentional dysfunction at both ages, with greater impairment in older trisomics. Density of BFCNs was significantly lower for Ts65Dn mice independent of age, which may contribute to attentional dysfunction since BFCN density was positively associated with performance on an attention task. BFCN volume decreased with age in 2N but not Ts65Dn mice. Paradoxically, BFCN volume was greater in older trisomic mice, suggestive of a compensatory response. In sum, attentional dysfunction occurred in both young and middle-aged Ts65Dn mice, which may in part reflect reduced density and/or phenotypic alterations in BFCNs.

Keywords Down syndrome · Aging · Attention · Basal forebrain cholinergic neurons · Choline acetyltransferase · Trisomic mice

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Introduction

Down syndrome (DS) is the most common known cause of intellectual disability, affecting 1 in approximately 800–1000 births (Centers for Disease Control and Prevention 2006; Parker et al. 2010). This disorder is caused by triplication of human chromosome 21 (HSA21) due to nondisjunction during meiosis. Individuals with DS generally develop dementia by the third decade of life (Lai and Williams 1989; Mann 1988; Visser et al. 1997; Wisniewski et al. 1985a, b), which coincides with the onset of Alzheimer's disease (AD)-like neuropathology, including senile plaques, neurofibrillary tangles (Wisniewski et al. 1985a) and atrophy/reduction of basal forebrain cholinergic neurons (BFCNs) (Casanova et al. 1985; Isacson et al. 2002; Mann et al. 1980; Sendera et al. 2000; Whitehouse et al. 1982).

A hallmark of DS is attentional dysfunction evidenced by shorter and fewer periods of sustained attention relative to developmentally age-matched controls (Brown et al. 2003). Longitudinal studies have reported deficits in selective and sustained attention during childhood, which is more pronounced during adolescence and adulthood in children with DS (Brown et al. 2003; Clark and Wilson 2003; Cornish et al. 2007; Tomporowski et al. 1990;

Wilding et al. 2002). The development of AD-like neuropathology during the third to fourth decade of life may contribute to this progressive attentional impairment in DS (Della Sala et al. 1992; Foster 2001; Krinsky-McHale et al. 2008; Levinoff et al. 2004; Pignatti et al. 2005). Although it has been hypothesized that the attentional dysfunction seen after the onset of AD-like neuropathology may be in part due to degeneration of BFCNs located within the nucleus basalis of Meynert/substantia innominata (NBM/SI) (Krinsky-McHale et al. 2008; Sarter and Bruno 1997), there is currently no evidence to support this link.

Several mouse models have been developed to study the relationship between the triplication of specific genes in DS and distinct phenotypic features (Das and Reeves 2011; Rueda et al. 2012; Salehi et al. 2006). The Ts65Dn mouse, a well-characterized model of DS and AD (Davisson et al. 1990; Holtzman et al. 1996), is trisomic for a segment of mouse chromosome 16 and mouse chromosome 17, with over 100 highly conserved genes that are orthologous to those on HSA21 (Mural et al. 2002; Patterson and Costa 2005; Sturgeon and Gardiner 2011). Ts65Dn mice survive to adulthood and exhibit many morphological, biochemical, and transcriptional changes similar to that seen in the human disorder (Antonarakis et al. 2001; Capone 2001; Davisson et al. 1990; Davisson et al. 1993; Holtzman et al. 1996; Reeves et al. 1995). Notably, these mice show deficiencies in developmental neurogenesis and hypocellularity (Bianchi et al. 2010; Guidi et al. 2011), which may play a role in their observed cognitive deficits (Abrous et al. 2008; Aimone et al. 2006; Leuner et al. 2006; Lledo et al. 2006; Shors et al. 2001, 2002). Furthermore, Ts65Dn mice are born with intact BFCNs, but the cholinergic neurons located within the medial septal nucleus (MSN) display atrophy at approximately 4–6 months of age (Cooper et al. 2001; Granholm et al. 2000; Holtzman et al. 1996). Ts65Dn mice show impairments in explicit memory and spatial mapping, subserved by the cholinergic MSN projections to the hippocampus (Ash et al. 2014; Granholm et al. 2000; Hyde and Crnic 2001; Hyde et al. 2001; Velazquez et al. 2013). These trisomic mice also exhibit impaired attention (Driscoll et al. 2004; Moon et al. 2010), a functional domain modulated by NBM/SI projections to the neocortex.

Our group previously reported attentional dysfunction in middle-aged (Moon et al. 2010) and aged (Driscoll et al. 2004) Ts65Dn mice. Similar to the attention deficits seen in individuals with DS (Krinsky-McHale et al. 2008), Ts65Dn mice were impaired on a series of visual attention tasks and were more frequently "off-task" compared to age-matched disomic (2N) controls (Driscoll et al. 2004; Moon et al. 2010). These impairments may reflect dysfunction of BFCNs in the NBM/SI, which display reduced numbers in DS (Casanova et al. 1985; Sendera et al. 2000) and AD

(Casanova et al. 1985; Mann et al. 1986; Mufson et al. 2000). Although we reported cholinergic neuron pathology in the NBM/SI and the medial septum/vertical limb of the diagonal band (MS/VDB) in young Ts65Dn mice (Kelley et al. 2014a, b), no structural or functional studies have detailed alterations in the NBM/SI and MS/VDB cholinergic neurons in relation to attentional dysfunction or aging in Ts65Dn mice.

The current study was designed to assess attentional function, BFCN morphology and their relationship in young (3 months) and middle-aged (12 months) Ts65Dn and 2N mice. Following the completion of a series of visual attention tasks as described previously by our group (Driscoll et al. 2004; Moon et al. 2010) animals were euthanized, brains processed for choline acetyltransferase (ChAT) immunohistochemistry, and BFCNs in the NBM/SI and MS/VDB were analyzed using quantitative unbiased stereology. Correlational analyses were also conducted to explore the relationship between task performance and BFCN morphometric data.

Methods

Subjects

Male Ts65Dn and age-matched 2N controls were purchased from Jackson Laboratories (Bar Harbor, ME) and raised at Cornell University (Ithaca, NY). Before being shipped to Cornell University for behavioral testing, the mice were genotyped via quantitative polymerase chain reaction for the detection of the extra chromosomal segment and determination of Pde6Brd1 homozygosity, a recessive mutation leading to retinal degeneration (Bowes et al. 1993). Pde6Brd1 homozygous mice were excluded from the study.

Young mice (referred to as 2N-Y and Ts65Dn-Y) were received at an average of 2 months of age whereas the older mice (2N-O and Ts65Dn-O) arrived at an average of 11 months of age. Upon arrival, the mice ($n = 16$/group) were housed individually in polycarbonate cages with food (AIN-76A; Dyets Inc., Bethlehem, PA) and water ad libitum. Mice were singly housed to prevent fighting between cagemates, which often occurs when group-housed male mice of this strain are returned to the home cage following daily behavioral testing. A combination of daily handling, testing and the provision of items in the home cage (i.e., plastic igloos, tubes, Nylabones®, and Nestlets) were designed to counter the environmental impoverishment of single-animal housing. The mice were maintained on a 12:12 reversed light dark cycle under temperature- and humidity-controlled conditions. All protocols were approved by the Institutional Animal Care and Use

Committee of Cornell University and conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Food restriction

One week after arrival, the mice were put on a food-restriction regimen to ensure motivation for food rewards (Liquefied AIN-76A; Bio-Serv, Frenchtown, NJ). Initially, mice were provided a daily ration of chow equivalent to the average daily amount (g) consumed since arrival (approximately 2–3 g/day). Throughout behavioral testing, the daily ration was determined on an individual basis, with the goal of feeding each mouse the maximal amount of food at which it would complete 60–70 trials in each daily test session. This approach was used to determine the daily food intake, rather than identifying a target weight (a fixed percentage of the starting weight), because the younger animals were still growing at the onset of behavioral testing. All animals were fed 15 min after each daily test session. The amount of chow to be fed was determined by subtracting the number of calories obtained as a reward in the testing chamber from the daily ration.

Behavioral testing

The testing apparatus has been previously described in our earlier publications (Driscoll et al. 2004; Moon et al. 2010). Briefly, mice were tested individually in 1 of 8 computer-controlled chambers. One wall contained a retractable metal door, controlling access to a retractable dipper that dispensed the liquid reward. The opposite wall contained five nose-poke response ports, each with a green 4 mA LED embedded on the back surface.

At the onset of behavioral testing, the young animals were approximately 3 months of age and the older animals were approximately 12 months of age. Each animal was pseudo-randomly assigned to a chamber, with each chamber balanced across the four treatment conditions defined by genotype and age. During testing on the various learning and attention tasks (described below), the animals received 1 test session per day, 6 days per week. Each daily session was terminated after 30 min or 70 trials, whichever came first. The entire testing series required 4–5 months to complete. Experimenters blind to genotype and age of the animals conducted all testing. Testing equipment was thoroughly cleaned and dried following the testing of each mouse, using Odormute (R.C. Steele Co, Brockport, NY).

Training

Training consisted of a series of four stages (see Driscoll et al. 2004 for details) designed to familiarize the animals with the testing chambers and the sequence of responses necessary to complete a trial for the visual attention tasks. The mice learned to initiate a trial with a nose-poke into the dipper alcove (with the dipper retracted). After trial initiation, a nose-poke into any one of the five response ports would produce the delivery of 0.01 ml of the liquid diet (i.e., reward) in the dipper alcove. During the final training stage, each animal was required to respond for a fixed number of trials at each of the five response ports, to eliminate preferences or aversions to any of the ports.

Initial visual discrimination task and error types

In this task, one of the five port LEDs was illuminated following trial initiation. The port remained illuminated until the mouse made a nose-poke into one of the ports, or until 32 s elapsed. The mouse was rewarded for making a nose-poke into the illuminated (i.e., correct) port. The location of the visual cue was pseudo-randomized across trials; the number of cue presentations in each port was balanced for each daily session. A 1-s delay separated trial initiation and cue onset. This delay, termed the "turn-around time," allowed the mouse to turn around and orient toward the ports before cue illumination. Three types of errors were distinguished: (1) premature response: a nose-poke into any response port prior to cue onset; (2) inaccurate response: a nose-poke made following cue onset but to one of the non-illuminated ports; and (3) omission error: failing to respond to any response port within 5-s of cue light termination. A 5-s intertrial interval separated adjacent trials. All trials in which the mouse made an initiation poke into the dipper alcove (regardless of the outcome of the trial) were defined as response trials. Failures to initiate a trial within 60 s were scored as nontrials, and no cues were presented. A 5-s time-out period was imposed following a nontrial or an error. This time-out period was signaled by the illumination of a 3-W houselight on the ceiling of the chamber. Each mouse remained on this task until it reached a criterion of 80 % correct for 2 out of 3 consecutive sessions, each consisting of at least 50 response trials.

Attention tasks 1 and 2

The mice were subsequently tested on 2 variants of the initial visual discrimination task that were identical except for the duration of the cue illumination, which was shortened to 2 and 1-s, respectively, for the two tasks. These tasks tested attentional function and prepared the mice for the subsequent task, which was more attentionally demanding. Attention tasks 1 and 2 were administered for eight sessions each.

Attention task 3 (learning to wait for the cue)

This task was identical to the prior task except that the duration between trial initiation and cue onset varied pseudo-randomly across trials. The pre-cue delay varied between 0, 2, and 4-s, all added to the 1-s turn-around time. Cue illumination duration was constant at 1 s. If a response was made prior to cue onset (i.e., premature response), the trial was terminated and no cue was presented. As noted above, premature responses (as well as all types of errors) were followed by a 5-s time-out period, signaled by the illumination of a 3-W houselight on the ceiling of the chamber. The 3 pre-cue delays were presented pseudo-randomly, such that the number of presentations of each combination of pre-cue delay and response port (1–5) was balanced across each session. The mice were tested for 18 sessions on this task. The early sessions on this task tap the ability of the mice to learn to wait for the cue, whereas the later sessions provide a more pure index of inhibitory control and focused attention. Thus, this task examines learning, inhibitory control and focused attention.

Tissue preparation and immunohistochemistry

Upon completion of behavioral testing, mice were deeply anesthetized with ketamine (83 mg/kg)/xylazine (13 mg/kg) via intraperitoneal injection and perfused transcardially with 4 % paraformaldehyde (50 ml) in phosphate buffer (PB; 0.1 M; pH = 7.4). Ages at killing averaged 7.6 months for young and 16.4 months for old mice. Brains were extracted from the calvaria, post-fixed for 24 h in the same fixative and placed in a 30 % sucrose PB solution at 4 °C until sectioning. Each brain was cut in the coronal plane at 40 μm thickness, on a sliding freezing microtome into six adjacent series and stored at 4 °C in a cryoprotectant solution (30 % ethylene glycol, 30 % glycerol, in 0.1 M PB) until processing.

Immunohistochemistry was performed as previously described (Ash et al. 2014; Kelley et al. 2014a, b). Sections were immunolabeled with a goat polyclonal antibody for choline acetyltransferase (1:1000 dilution, Millipore, Billerica, MA). Tissue was washed in PB to remove excess cryoprotectant, rinsed in Tris-buffered saline (TBS), and incubated in sodium metaperiodate to inhibit endogenous peroxide activity. To enhance primary antibody penetrance, tissue was washed in TBS containing 0.25 % Triton X-100 followed by incubation in a blocking solution consisting of 3 % horse serum in TBS/Triton X-100. Tissue was then incubated overnight at room temperature with ChAT primary antibodies in a solution of TBS/Triton X-100 with 1 % serum. All washes and incubations were carried out at room temperature on a shaker plate. Following overnight incubation in primary antibody, sections were washed in TBS and incubated for 1 h with a biotinylated secondary antibody (anti-goat IgG, host horse; Vector Laboratories, Inc., Burlingame, CA). Sections were washed in TBS and were incubated for 1 h in avidin–biotin complex solution (Elite kit, Vector Laboratories, Inc.) to amplify signal. Tissue was then washed in a sodium imidazole acetate buffer, and antibody immunolabeling for ChAT was visualized using a chromogen solution consisting of 0.05 % 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO), 1 % nickel (II) ammonium sulfate hexahydrate, and 0.0015 % H_2O_2. Sections were washed in acetate-imidazole buffer to terminate reactions, mounted onto chrome alum-submersed slides, dried overnight at room temperature, dehydrated in a graded series of ethanol, cleared in xylenes, and coverslipped with distyrene/dibutylphthalate (plasticizer)/xylene (DPX) mounting medium. Antibody penetration was determined by evaluating tissue through Z-stacks using the stereology system as previously reported (Kelley et al. 2014a, b). Control sections were processed using the same procedures as above with the exception of incubation with the primary antibody.

Morphometric analysis

BFCN subregions examined included the MS/VDB and NBM/SI (Kelley et al. 2014a, b). In preliminary studies, we found no difference between MSN and VDB measures; so these regions were combined. Estimation of ChAT-immunoreactive (ir) neuron number was derived using the optical fractionator, a stereology system that pairs the optical disector probe (a three-dimensional counting space) with a two-dimensional grid that provides an unbiased random start and systematic interval sampling of the region of interest. All analyses were conducted using Stereo Investigator software (version 9.14.5 32-bit, MicroBright-Field, Inc., Williston, VT) coupled to a Nikon Optiphot-2 microscope, as reported previously (Ash et al. 2014; Kelley et al. 2014a, b). Values are presented as estimate per brain, derived from a sampling of the region of interest bilaterally across a 1/6 series (X60 n.a., 1.40, 50 × 50 μm counting frame, 151 × 151 μm grid size, 10 μm disector height). Tissue thickness was measured at every site that contained cells and the reciprocal for (disector height)/(mean measured thickness) was used for reported numbers and statistical analyses. The large sampling fraction allowed for a $CE_{m = 1}$ of ≤0.10 (Gundersen et al. 1999). Cell density is presented as cells per 1,000,000 μm³. Calculation was performed for each animal, prior to group averages.

BFCN volume was measured using a 5-ray nucleator probe for an average of 60 cells per stain, per region, per animal (X60 oil-immersion lens n.a. 1.40) using random sampling across rostrocaudal and dorsoventral axes derived

with the optical fractionator. The nucleator involves taking five measurements from an approximate center of the cell to the perimeter of the cell in one plane (<1.0-μm z axis) of section (Gundersen 1988). The probe derives an average radius for each cell and volume was calculated from this value using a weighted geometric formula (shape assumption spheroid).

Statistical analyses

Statistical analyses were performed using the Statistical Analysis System (Version 9.3; SAS Institute, Cary, NC). Fixed factors included genotype (Ts65Dn and 2N) and age (young and old). Attention task performance measures were analyzed using PROC GLM when trial conditions did not vary and performance was stable across testing sessions, and PROC GLIMMIX when the task involved a time (learning) component. PROC GLIMMIX is a generalized linear mixed models procedure for conducting repeated measures analyses. The dependent measures included: sessions to criterion, percentage of correct responses, percentage of omission errors, percentage of inaccurate responses, and percentage of premature responses. If a violation of homogeneity of variance was present, determined by the Levene test of homogeneity, a non-parametric analysis (i.e., Kruskal–Wallis test) was utilized. Some mice were excluded from analyses due to apparatus malfunction or insufficient motivation; the final sample size is presented with the figures.

The primary dependent measures for the morphometric data were the number, density, and volume of ChAT-ir cells within the NBM/SI and MS/VDB. These analyses were conducted using 2×2 ANOVAs. Some mice were excluded due to tissue labeling variability; the final sample size is presented with the figures.

Spearman's rank correlation coefficients were used to assess the relationships between percentage of correct responses in attention tasks 1 and 2 and number, density and volume of ChAT-ir cells within the NBM/SI and MS/VDB. Correlational analyses focused on these two tasks because performance tends to be stable across all test sessions and specifically reflects focused attention, in contrast to attention task 3, in which performance changes across sessions, and reflects learning and inhibitory control as well as attention. The correlational analyses were viewed as exploratory, and therefore, no corrections were made for multiple testing.

The alpha level was set at 0.05 for all analyses. Four a priori contrasts were conducted regardless of whether or not the interaction of genotype and age was significant (because they are central to the goals of the study). These four comparisons were: (1) 2N-Y vs. Ts56Dn-Y, (2) Ts65Dn-Y vs. Ts65Dn-O, (3) 2N-Y vs. 2N-O, and (4) 2N-

O vs. Ts65Dn-O. A Bonferroni correction was applied to these comparisons, resulting in an alpha level of 0.05/4 (0.0125) as the threshold for significance.

Results

Body weight

Analysis of body weight at the completion of behavioral testing revealed significant effects of genotype [$F_{(1, 34)} = 16.12$, $p = 0.0003$] and age [$F_{(1, 34)} = 18.31$, $p = 0.0001$]; the interaction of these variables was not significant [$F_{(1, 34)} = 3.52$, $p = 0.07$]. Ts65Dn mice (mean = 21.03 g; SEM = 0.63) weighed significantly less than 2N mice (mean = 23.6 g; SEM = 0.63). As expected, younger mice (mean = 20.95 g; SEM = 0.59) weighed less than older mice (mean = 23.6 g; SEM = 0.66). Body weight findings are consistent with prior reports (Ash et al. 2014; Bianchi et al. 2010; Fuchs et al. 2012; Velazquez et al. 2013).

Learning and attention

Five-choice visual discrimination task (no pre-cue delay, 32-s cue duration)

Sessions to criterion, the dependent measure for learning rate in the initial visual discrimination task, was analyzed using the nonparametric Kruskal–Wallis test and revealed a significant group effect [$H_{(3)} = 9.857$, $p = 0.02$] (Fig. 1a). Contrasts revealed a significant effect of age for the 2N mice, but not the Ts65Dn mice. Specifically, 2N-Y mice reached criterion significantly faster than the 2N-O mice ($p = 0.003$). 2N-Y mice also reached criterion in fewer sessions than the Ts65Dn-Y mice ($p = 0.04$), but this contrast was not significant following the Bonferroni correction. Learning rate was not affected by trisomy for the older animals.

Attention task 1 (no pre-cue delay, 2-s cue duration, eight sessions)

The analysis of percentage of correct responses for attention task 1 revealed significant main effects of genotype [$F_{(1, 34)} = 15.79$, $p = 0.0003$] and age [$F_{(1, 34)} = 10.50$, $p = 0.003$] reflecting poorer performance of Ts65Dn mice relative to 2N controls, and inferior performance of the older mice relative to the younger mice (Fig. 1b). The interaction of genotype and age was not significant [$F_{(1, 34)} = 1.33$, $p = 0.26$] indicating that the magnitude of impairment in trisomic mice (relative to 2N) was comparable at the two ages examined. Planned contrasts revealed a significant trisomy effect for younger mice. Specifically, Ts65Dn-Y mice
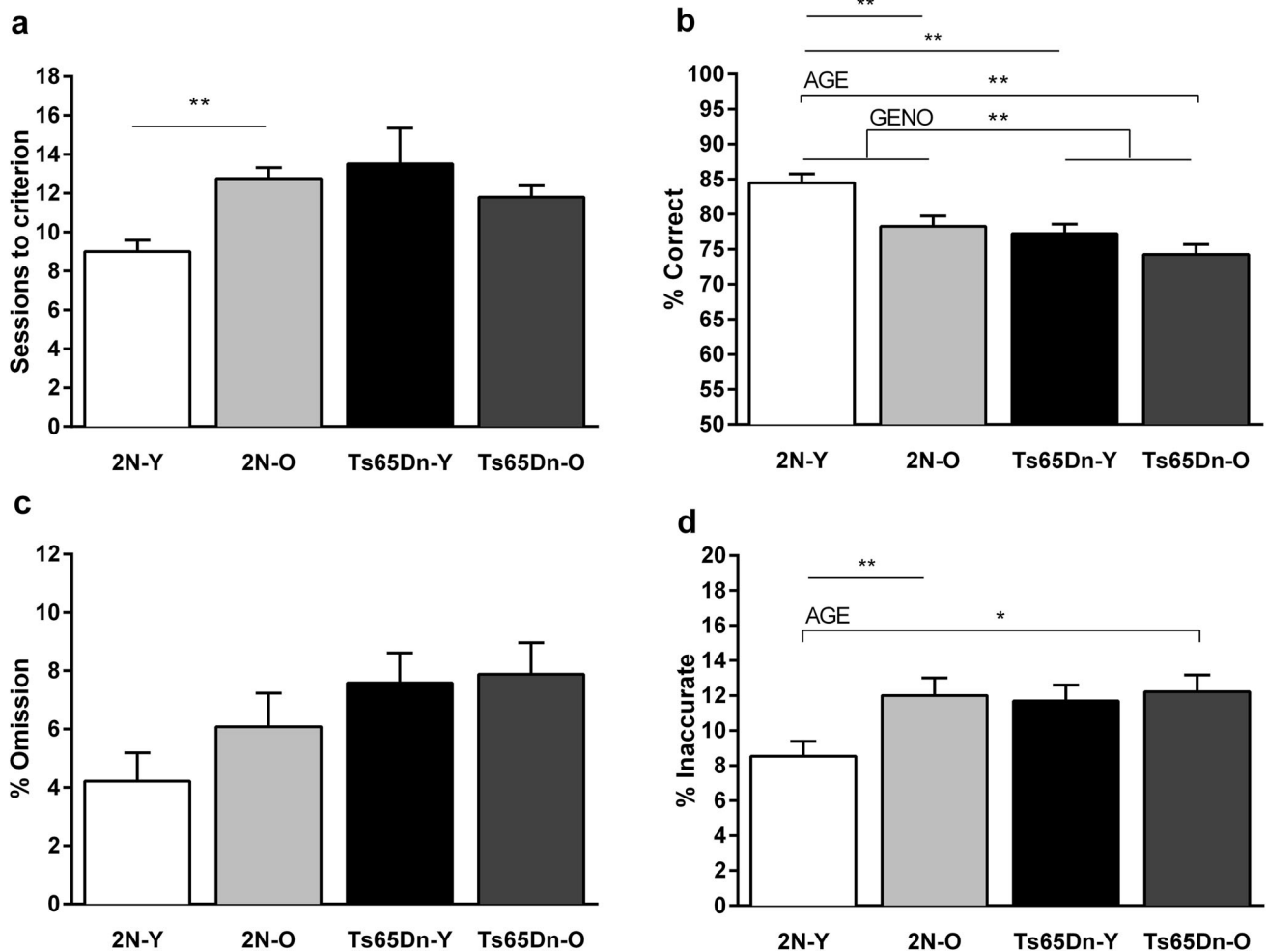
Fig. 1 Visual discrimination task and attention task 1. **a** Mean (±SEM) sessions to criterion in the initial visual discrimination task. 2N-Y mice reached criterion significantly faster than the 2N-O group ($p = 0.003$). **b** Mean (±SEM) percentage of correct responses in attention task 1. A significant main effect of genotype confirmed a performance deficit in Ts65Dn mice ($p = 0.0003$). A significant main effect of age indicated better performance in young mice ($p = 0.003$). Planned contrasts revealed significant impairment in Ts65Dn-Y compared to 2N-Y ($p = 0.001$), and a detrimental effect of aging in the 2N mice ($p = 0.004$). **c** Mean (±SEM) percentage of omission errors during attention task 1. Both trisomic groups and 2N-O mice committed a higher percentage of omission errors than 2N-Y mice, but contrasts did not meet significance following the Bonferroni correction. **d** Mean (±SEM) percentage of inaccurate responses during attention task 1. A significant main effect of age indicated a higher percentage of inaccurate responses for the older mice ($p = 0.04$). Planned contrasts revealed an effect of aging for the 2N mice ($p = 0.01$). 2N-Y $n = 11$, 2N-O $n = 8$, Ts65Dn-Y $n = 10$, Ts65Dn-O $n = 9$. *$p \leq 0.05$, **$p \leq 0.01$

were significantly impaired relative to the 2N-Y mice ($p = 0.001$). In addition, a significant detrimental effect of aging was seen for the 2N-O mice ($p = 0.004$). No other comparisons were significant.

Percentage of omission errors analyzed using the non-parametric Kruskal–Wallis test uncovered a significant effect of group [$H(3) = 8.54$, $p = 0.03$]. However, none of the planned contrasts were significant following the Bonferroni correction (Fig. 1c).

Analysis of percentage of inaccurate responses revealed a significant main effect of age [$F(1, 34) = 4.58$,

$p = 0.04$], reflecting a higher percentage of inaccurate responses for the older mice (Fig. 1d). Neither the main effect of genotype [$F(1, 34) = 3.28$, $p = 0.08$] nor the interaction of genotype × age [$F(1, 34) = 2.48$, $p = 0.12$] was statistically significant. Planned contrasts revealed a detrimental effect of aging for the 2N mice ($p = 0.01$). In addition, the Ts65Dn-Y mice committed a higher percentage of inaccurate responses than their 2N-Y controls ($p = 0.02$), but this contrast was not significant following the Bonferroni correction. No other group comparisons were significant.

Attention task 2 (no pre-cue delay, 1-s cue duration, eight sessions)

Analysis of percentage of correct responses revealed significant main effects of genotype [$F_{(1, 37.29)} = 12.68$, $p = 0.001$] and age [$F_{(1, 37.29)} = 9.48$, $p = 0.004$], as well as a significant interaction of these two variables [$F_{(1, 37.29)} = 4.09$, $p = 0.05$] (Fig. 2a). Contrasts revealed that Ts65Dn-O mice performed significantly worse than their younger counterparts ($p = 0.001$). Ts65Dn-O mice were also significantly impaired relative to 2N-O controls ($p = 0.005$). There was no effect of age in 2N mice, and there was no effect of genotype within younger mice.

An analysis of percentage of omission errors revealed a significant main effect of genotype [$F_{(1, 32)} = 33.69$, $p < 0.0001$] as well as a significant genotype × age interaction [$F_{(1, 32)} = 6.01$, $p = 0.02$] (Fig. 2b). Contrasts revealed that Ts65Dn-O mice made a significantly higher percentage of omission errors than their younger counterparts ($p = 0.008$) and also their age-matched 2N controls ($p < 0.0001$). The analysis of percentage of inaccurate responses did not reveal significant effects of age, genotype, or the interaction of these two variables (data not shown).

Attention task 3 (0-, 2-, or 4-s variable pre-cue delay; 1-s cue duration; 18 sessions)

Analysis of the percentage of correct responses for attention task 3 revealed a significant effect of pre-cue delay [$F_{(2, 302.7)} = 605.07$, $p < 0.0001$], reflecting declining performance with increasing pre-cue delay. Session block (6 blocks of 3 sessions each) was also significant [$F_{(5, 208.8)} = 154.73$, $p < 0.0001$], indicating improvement in performance with continued training. There was neither effect of age nor any significant interactions with age. A main effect of genotype was seen [$F_{(1, 40.21)} = 5.30$, $p = 0.03$] as well as a significant genotype × delay × session block interaction [$F_{(10, 300.7)} = 3.71$, $p = 0.0001$] (Fig. 3). Contrasts revealed that on trials with a 0-s pre-cue delay (Fig. 3a), Ts65Dn mice performed significantly worse than the 2N mice during blocks 1 ($p = 0.001$), 2 ($p = 0.04$), 3 ($p = 0.04$), 4 ($p = 0.05$), and 6 ($p = 0.02$). On trials with a 2-s pre-cue delay (Fig. 3b), Ts65Dn mice performed worse than 2N mice during blocks 3 ($p = 0.01$), 4 ($p = 0.002$), and 5 ($p = 0.04$). Analysis of trials with a 4-s pre-cue delay (Fig. 3c) revealed that Ts65Dn mice performed significantly better than 2N mice during block 1 ($p = 0.03$), but worse than 2N mice during blocks 4 ($p < 0.0001$) and 5 ($p = 0.002$), with similar trends during blocks 3 ($p = 0.06$) and 6 ($p = 0.07$).

In the analysis of percentage of premature responses, a highly significant effect of pre-cue delay was seen [$F_{(1, 223.6)} = 457.64$, $p < 0.0001$], reflecting the fact that the percentage of premature responses increased as the interval prior to cue presentation increased. Session block was also highly significant [$F_{(5, 121.5)} = 82.96$, $p < 0.0001$], indicating a decrease in the percentage of premature responses with continued training, as the animals learned to wait for the cue. There was also a genotype × session block interaction [$F_{(5, 121.5)} = 4.23$, $p = 0.001$] reflecting that 2N mice committed a higher percentage of premature responses than
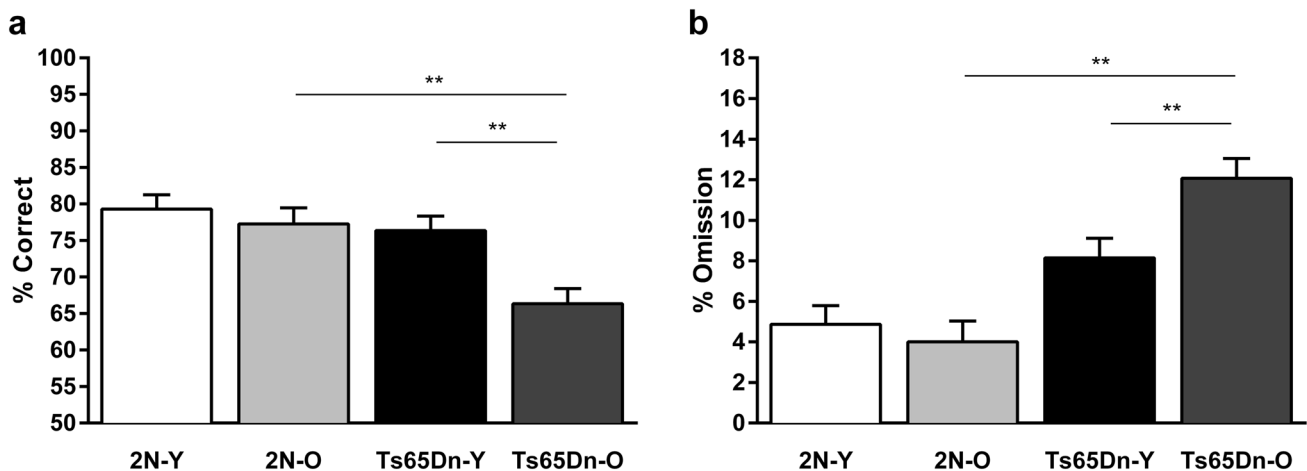

a

b

Fig. 2 Attention task 2. **a** Mean (±SEM) percentage of correct responses. A genotype × age interaction revealed that for the older mice, the Ts65Dn mice performed more poorly than their 2N counterparts ($p = 0.005$), whereas no genotype effect was seen for the young mice. Contrasts also revealed a significant effect of aging in the Ts65Dn mice ($p = 0.001$) but not the 2N. **b** Mean (±SEM) percentage of omission errors. Similarly, a significant genotype × age interaction revealed a significant effect of the trisomy for the old mice ($p < 0.0001$), but not the young mice, and a significant effect of aging for the Ts65Dn mice ($p = 0.008$), but not the 2N. 2N-Y $n = 10$, 2N-O $n = 8$, Ts65Dn-Y $n = 10$, Ts65Dn-O $n = 9$. *$p \leq 0.05$, **$p \leq 0.01$
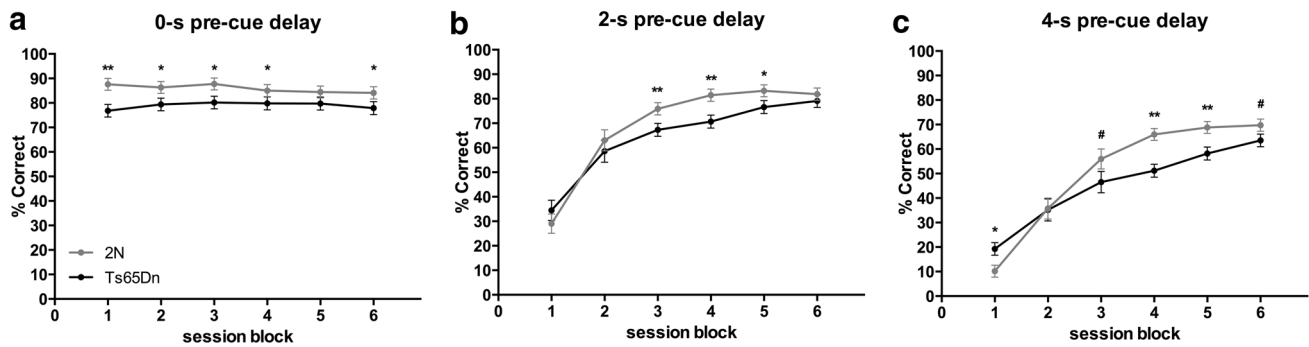
Fig. 3 Mean (±SEM) percentage of correct responses in attention task 3 as a function of session block at each duration of the pre-cue delay. **a** Mean (±SEM) percentage of correct responses on trials with a 0-s pre-cue delay. Ts65Dn mice performed worse than 2N mice during blocks 1 ($p = 0.001$), 2 ($p = 0.04$), 3 ($p = 0.04$), 4 ($p = 0.05$), and 6 ($p = 0.02$). **b** Mean (±SEM) percentage of correct responses on trials with a 2-s pre-cue delay. Ts65Dn mice performed

worse than 2N mice during blocks 3 ($p = 0.01$), 4 ($p = 0.002$), and 5 ($p = 0.04$). **c** Mean (±SEM) percentage of correct responses on trials with a 4-s pre-cue delay. Ts65Dn mice performed worse than the 2N during blocks 4 ($p < 0.0001$) and 5 ($p = 0.002$), with similar trends during blocks 3 ($p = 0.06$) and 6 ($p = 0.07$), whereas the opposite pattern was seen for block 1 ($p = 0.01$). 2N $n = 19$, Ts65Dn $n = 17$. *$p \leq 0.05$, **$p \leq 0.01$, #$p \leq 0.07$
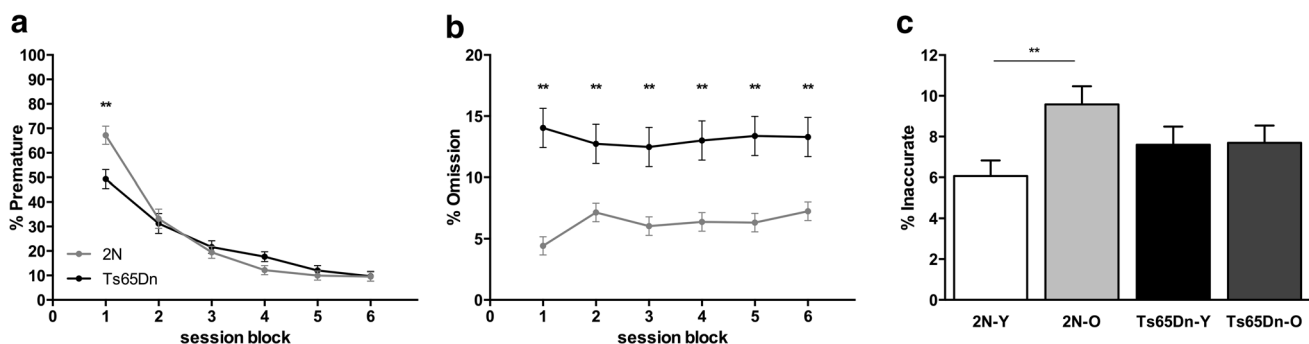

Fig. 4 Attention task 3. **a** Mean (±SEM) percentage of premature responses. A significant genotype × session block interaction revealed genotype differences only during block 1, where the 2N mice committed a higher percentage of premature responses than Ts65Dn mice ($p = 0.002$). **b** Mean (±SEM) percentage of omission errors. Ts65Dn mice committed a higher percentage of omission

errors than the 2N mice across all session blocks ($p \leq 0.01$). **c** Mean (±SEM) percentage of inaccurate responses. A significant genotype × age interaction revealed that aging increased the percentage of inaccurate responses for 2N mice ($p = 0.005$) but not trisomics. 2N-Y $n = 11$, 2N-O $n = 8$, Ts65Dn-Y $n = 9$, Ts65Dn-O $n = 8$. *$p \leq 0.05$, **$p \leq 0.01$

Ts65Dn mice only during the first block ($p = 0.002$) (Fig. 4a). Genotype did not impact the percentage of premature responses throughout the remaining session blocks.

Analysis of percentage of omission errors revealed a significant effect of pre-cue delay [$F (2, 86.24) = 7.91$, $p = 0.001$]. Omission errors increased with longer pre-cue delays. A highly significant effect of genotype was seen [$F (1, 19.49) = 18.98$, $p = 0.0003$], as well as a significant genotype × block interaction [$F (5, 517.3) = 2.73$, $p = 0.02$]. Across all session blocks, trisomic mice exhibited a substantially greater incidence of omission errors, with the size of the effect greatest during block 1 (Fig. 4b). There was no significant main effect of age for percentage of omission errors [$F (1, 19.49) = 0.02$, $p = 0.89$] nor significant interactions involving age.

In the analysis of percentage of inaccurate responses, a significant effect of age was seen [$F (1, 32.07) = 4.54$,

$p = 0.04$] as well as a genotype × age interaction [$F (1, 32.07) = 4.06$, $p = 0.05$] (Fig. 4c). Contrasts revealed a significant detrimental effect of aging for this measure for 2N mice ($p = 0.005$), but not for Ts65Dn mice.

Quantitative morphometric analysis of ChAT-ir cells

ChAT-ir neuron number in the NBM/SI

Analysis of the number of ChAT-ir cells in the NBM/SI did not reveal a main effect of genotype [$F (1, 55) = 0.80$, $p = 0.4$] (Fig. 5a) nor a genotype × age interaction [$F (1, 55) = 1.44$, $p = 0.24$]. There was a significant main effect of age [$F (1, 55) = 6.53$, $p = 0.01$], revealing a 16.6 % decrease in ChAT-ir neuron number in older mice compared to their younger counterparts. Planned contrasts
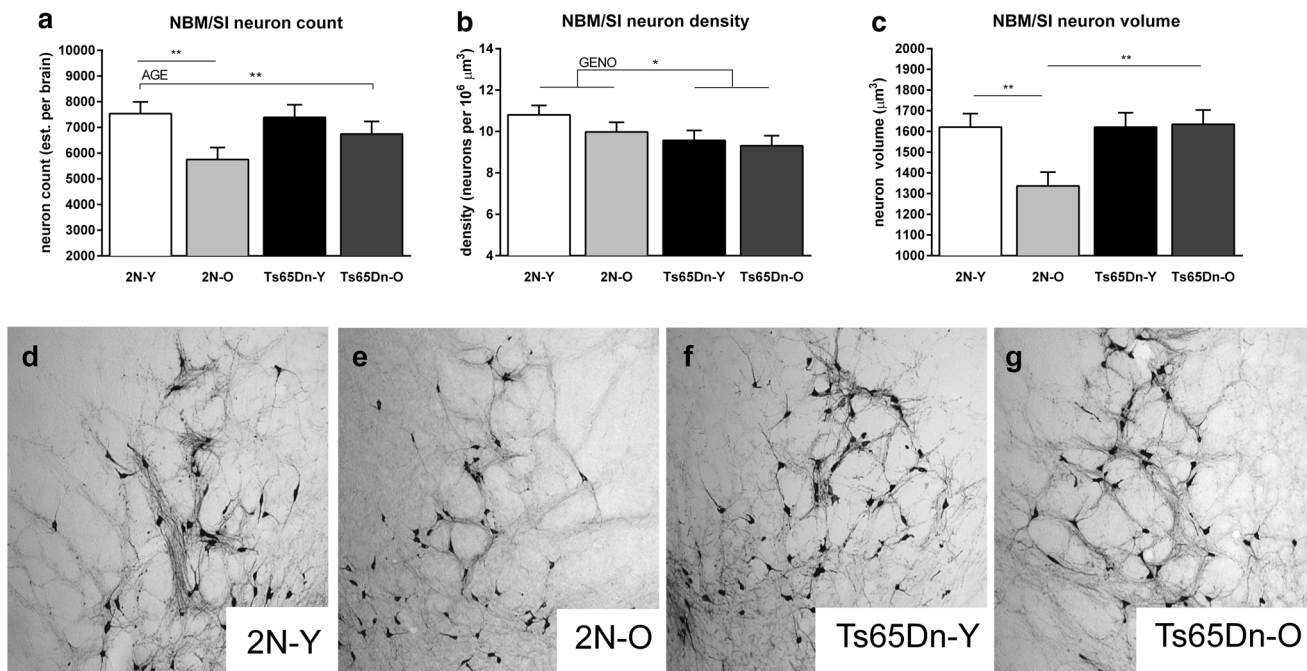
Fig. 5 Morphometric indices of ChAT-ir neurons in the NBM/SI. **a** Mean (±SEM) neuron count in the NBM/SI. A main effect of age indicated that older mice exhibited fewer neurons than younger mice ($p = 0.01$). Planned contrasts revealed a significant reduction in 2N-O mice compared to their younger counterparts ($p = 0.01$). **b** Mean (±SEM) neuron density in the NBM/SI. A significant main effect of genotype reflected reduced density in the trisomics ($p = 0.05$). **c** Mean

(±SEM) neuron volume in the NBM/SI. A significant interaction of genotype × age reflected the fact that aging decreased neuron size for 2N ($p = 0.004$) but not trisomic mice. Accordingly, 2N-O mice had smaller ChAT-ir neurons than Ts65Dn-O mice ($p = 0.003$). **d– g** Representative photomicrographs of ChAT-ir neurons in the NBM/ SI (100x magnification). 2N-Y $n = 16$, 2N-O $n = 14$, Ts65Dn-Y $n = 13$, Ts65Dn-O $n = 14$. #$p = 0.06$, *$p \leq 0.05$, **$p \leq 0.01$

revealed a significant reduction in ChAT-ir neuron number in 2N-O relative to 2N-Y mice ($p = 0.01$) (Fig. 5a). The remaining contrasts were not significant.

ChAT-ir neuron density in the NBM/SI

A significant effect of genotype was found for ChAT-ir neuron density within the NBM/SI [$F (1, 55) = 3.99$, $p = 0.05$] (Fig. 5b). Ts65Dn mice showed a significant 9.72 % reduction relative to 2N controls. No significant effect of age was detected [$F (1, 55) = 1.31$, $p = 0.26$] nor was there an interaction of genotype and age [$F (1, 55) = 0.36$, $p = 0.55$].

ChAT-ir neuron volume in the NBM/SI

Analysis of ChAT-ir neuron volume within the NBM/SI revealed significant effects of genotype [$F (1, 55) = 4.48$, $p = 0.03$], age [$F (1, 55) = 3.99$, $p = 0.05$] and geno-type × age interaction [$F (1, 55) = 4.87$, $p = 0.03$] (Fig. 5c). Contrasts revealed that for 2N mice, advancing age resulted in a reduced size of ChAT-ir neurons ($p = 0.003$), whereas no effect of age was seen for Ts65Dn mice. Consequently, ChAT-ir neurons in this region were significantly larger in Ts65Dn-O compared to 2N-O mice ($p = 0.003$).

ChAT-ir neuron number in the MS/VDB

Analysis of ChAT-ir neuron counts within the MS/VDB did not reveal a main effect of genotype [$F (1, 55) = 0.14$, $p = 0.71$] (Fig. 6a) nor a significant genotype × age interaction [$F (1, 55) = 0.95$, $p = 0.33$]. However, the effect of age was significant [$F (1, 55) = 5.73$, $p = 0.02$], revealing a 16.6 % decrease in older mice relative to their younger counterparts for both genotypes.

ChAT-ir neuron density in the MS/VDB

Analysis of ChAT-ir neuron density in the MS/VDB revealed a significant effect of age, indicating a 14.3 % decrease in the older mice [$F (1, 55) = 4.75$, $p = 0.03$] (Fig. 6b). A borderline genotype effect [$F (1, 55) = 3.52$, $p = 0.06$] was seen with 12.9 % lower ChAT-ir neuron density for Ts65Dn relative to 2N mice. There was no genotype × age interaction for this measure within the MS/VDB [$F (1, 55) = 1.02$, $p = 0.32$].

ChAT-ir neuron volume in the MS/VDB

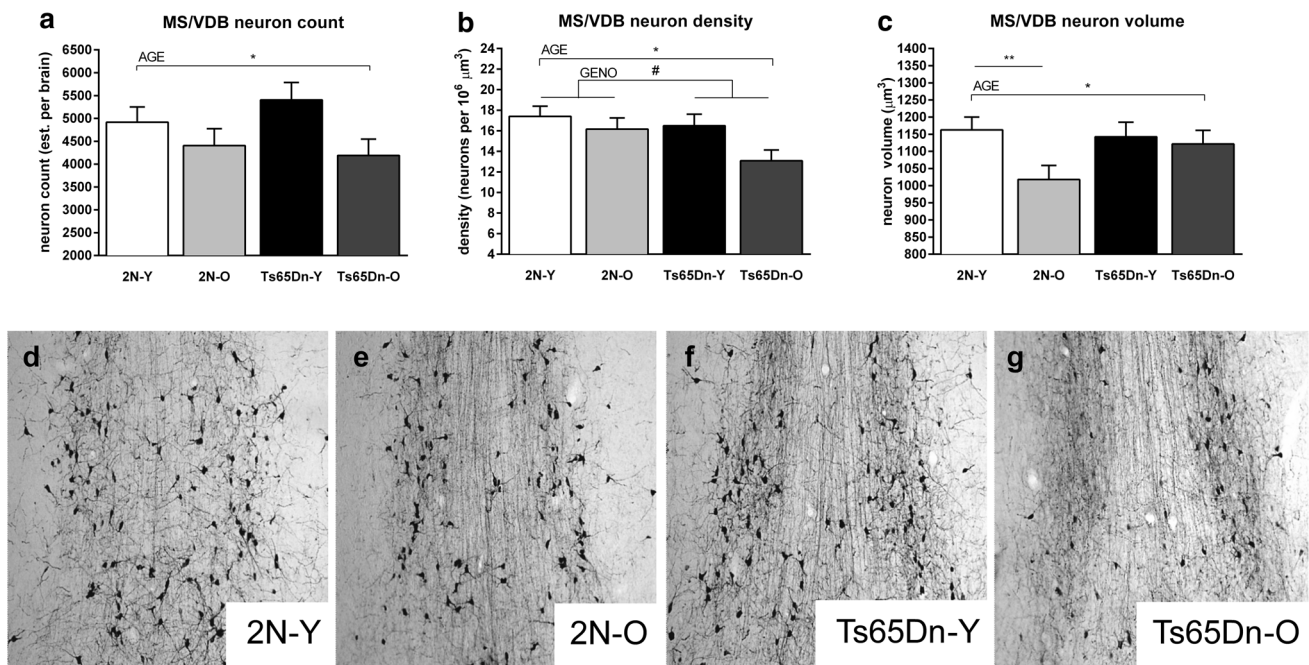Analysis of ChAT-ir neuron volume in the MS/VDB revealed a main effect of age [$F (1, 55) = 4.25$, $p = 0.04$],

Fig. 6 Morphometric indices of ChAT-ir neurons in the MS/VDB. **a** Mean (±SEM) neuron count in the MS/VDB. A main effect of age indicated that older mice exhibited fewer neurons than younger mice ($p = 0.02$). **b** Mean (±SEM) neuron density in the MS/VDB. A main effect of age indicated that older mice exhibited a reduced density compared to younger mice ($p = 0.03$). A borderline effect of genotype reflected reduced density in Ts65Dn mice ($p = 0.06$). **c** Mean (±SEM) neuron volume in the MS/VDB. A main effect of age indicated that older mice had a reduced neuron size compared to younger animals ($p = 0.03$). Planned comparisons revealed that older 2N mice exhibit decreased neuron size compared to their younger counterparts ($p = 0.01$), similar to that seen in the NBM/SI. **d–g** Representative photomicrographs of ChAT-ir neurons in the MS/VDB (100x magnification). 2N-Y $n = 16$, 2N-O $n = 14$, Ts65Dn-Y $n = 13$, Ts65Dn-O $n = 14$. #$p = 0.06$, *$p \leq 0.05$, **$p \leq 0.01$

but no effect of genotype [$F (1, 55) = 1.08$, $p = 0.32$] (Fig. 6c). Although the interaction of genotype and age was not significant [$F (1, 55) = 2.38$, $p = 0.13$], planned contrasts revealed that age did not alter neuron volume for the Ts65Dn mice. However, the 2N-O mice exhibited a 12.45 % reduction in neuron volume relative to their younger counterparts ($p = 0.01$).

Correlations between percentage of correct responses and ChAT-ir neuron morphometrics in the NBM/SI and MS/VDB

Correlational analyses uncovered three significant associations between ChAT-ir BFCNs and attention task performance (Table 1). All of these were observed in the older mice (2N and Ts65Dn combined), but not the younger mice, as will be discussed below. Specifically, within the older mice, there was a significant positive association between percentage of correct responses on attention task 1 and density of ChAT-ir neurons in both the NBM/SI ($r_s = 0.581$, $p = 0.03$; Fig. 7a) and MS/VDB ($r_s = 0.559$, $p = 0.03$; Fig. 7b). Density of ChAT-ir neurons in these two regions was not significantly correlated with performance on attention task 2. There was also a significant

Table 1 Correlations between percentage correct on attention tasks 1 and 2 and BFCN morphometrics in the NBM/SI and MS/VDB (includes only 2N-O and Ts-O mice)

	Count	Density	Volume	Count	Density	Volume
Attention task 1						
r_s	0.286	**0.581**	0.068	0.207	**0.559**	0.145
p	0.32	**0.03***	0.82	0.46	**0.03***	0.61
Attention task 2						
r_s	−0.231	0.029	**−0.541**	0.232	0.291	−0.243
p	0.43	0.92	**0.04***	0.40	0.29	0.38

* $p \leq 0.05$

negative association between percentage of correct responses on attention task 2 and ChAT-ir neuron volume in the NBM/SI ($r_s = −0.541$, $p = 0.04$; Fig. 7c).

Discussion

Effects of trisomy in young mice

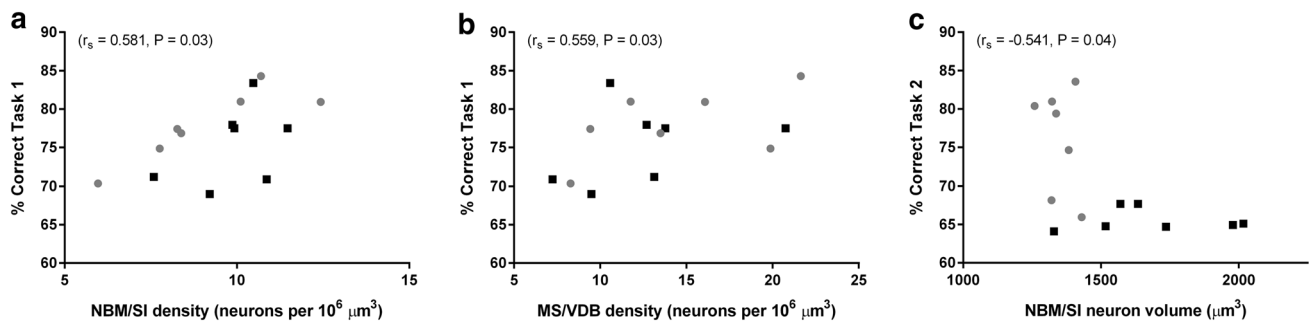The present findings demonstrate that young Ts65Dn mice exhibit impairments in both learning and attention. These

Fig. 7 Correlations between attention task performance and BFCN morphometrics in the old mice (genotypes combined). **a** A significant positive correlation was seen between percentage of correct responses in attention task 1 and ChAT-ir neuron density in the NBM/SI ($r_s = 0.581$, $p = 0.03$); i.e., as ChAT-ir neuron density increased, percentage of correct responses increased. **b** A similar pattern was observed for ChAT-ir neuron density in the MS/VDB ($r_s = 0.559$, $p = 0.03$). **c** A significant negative correlation was observed between percentage of correct responses in attention task 2 and ChAT-ir neuron volume in the NBM/SI ($r_s = -0.541$, $p = 0.04$). 2N-O (*gray circles*) $n = 7$, Ts65Dn-O (*black squares*) $n = 7$

mice tended to require more sessions to reach criterion for the initial visual discrimination task than age-matched 2N controls, indicative of impaired associative ability. Notwithstanding, trisomic mice eventually reached criterion on this task and performed as well as controls. The high level of performance of these mice after learning the task rules excludes deficits in visual acuity, motoric function, or motivation as the basis for their impaired performance in subsequent tasks.

Ts65Dn-Y mice performed worse than 2N-Y mice on attention task 1. Since the Ts65Dn-Y and 2N-Y mice did not differ in their performance level by the end of the initial visual discrimination task, their poorer performance during testing on attention task 1 can be attributed to the reduced duration of the visual cue, which places a greater demand on focused attention. The inference of attentional dysfunction is supported by the pattern of results on attention task 3. In this task, the Ts65Dn mice of both ages performed worse than the 2N. This task was the first to include a variable delay prior to presentation of the cue, but the poorer performance of the trisomic mice compared to 2N controls was not due to an increased incidence of premature responses (responses made prior to cue onset). Both genotypes made a high number of premature responses during the first session block, but significantly reduced these errors across subsequent blocks as they learned to wait for cue presentation. The performance deficit in Ts65Dn mice was mainly due to an increased incidence of omission errors, which was seen across all session blocks. Indeed, the most notable difference between genotypes on all three tasks was the higher percentage of omission errors observed in Ts65Dn mice. This is consistent with a prior study from our lab, in which videotape analyses revealed that Ts65Dn mice attended to the response ports less than controls in the period between trial initiation and cue presentation, and therefore were more likely to miss the

cue (Driscoll et al. 2004). The observation that trisomics did not differ from 2N mice in trial initiation (substantiating equal motivation), but then more frequently failed to respond following cue presentation (indicative of missing the cue), implicates attentional dysfunction. These results demonstrate that Ts65Dn-Y mice, like their older counterparts, are impaired in tasks requiring attentional focus during a pre-cue delay. This finding is consistent with the human literature showing that children and young adults with DS are impaired in sustained attention (Brown et al. 2003; Cornish et al. 2007; Tomporowski et al. 1990; Wilding et al. 2002), providing a model system that may be used for therapeutic intervention studies with direct relevance to human DS.

Effects of trisomy in old mice

Aged Ts65Dn mice, tested between 12 and 16 months of age, did not differ from their 2N counterparts in rate of learning the initial visual discrimination task. When the cue duration in attention task 1 was shortened to 2-s, there was no detectable difference between Ts65Dn-O and 2N-O mice. However, when the cue duration was further reduced to 1 s in attention task 2, the Ts65Dn-O mice performed significantly worse than their 2N counterparts. This impairment was due to an increased percentage of omission errors, indicative of missing the cue. When the mice were further challenged with a variable pre-cue delay in attention task 3, trisomics were impaired relative to the 2N mice, again due primarily to an increased incidence of omission errors by the trisomic mice. The fact that these mice did not differ from their 2N counterparts in the initial visual discrimination or attention task 1 indicates that they were not impaired in the visual or motoric demands of the task, nor in motivation or understanding task rules. Rather, this pattern of increased omission errors, coupled with an

absence of group differences in premature responses, implicates attentional dysfunction, consistent with previous observations (Driscoll et al. 2004; Moon et al. 2010). In a prior study, analysis of videotapes of mice performing these attention tasks revealed that aged trisomics were frequently "off-task" during the pre-cue delay, exploring the side of the chamber opposite to the ports (Driscoll et al. 2004). Taken together, these behavioral data suggest that aged Ts65Dn mice are impaired relative to age-matched 2N controls in tasks that require focused or sustained attention.

Effects of aging in Ts65Dn mice

We hypothesized that attentional dysfunction in Ts65Dn mice would be exacerbated with aging. This was the case for some, but not all tasks. Ts65Dn-O mice did not differ from Ts65Dn-Y mice in attention task 1, in which the duration of cue illumination was relatively brief (2-s). However, when cue duration was further decreased to 1 s in attention task 2, placing even greater demands on attention, performance of Ts65Dn-O mice was significantly worse than Ts65Dn-Y mice. The decreased percentage of correct responses seen in older trisomic mice relative to their younger counterparts was driven by an increased percentage of omission errors. Consistent with our previous observations (Driscoll et al. 2004), this pattern implicates impaired focused attention and/or a greater incidence of being off-task, which progressively worsens with age in Ts65Dn mice. In attention task 3, both young and old Ts65Dn mice were impaired relative to 2N controls, driven primarily by an increased incidence of omission errors. These findings suggest that Ts65Dn-O mice are impaired relative to Ts65Dn-Y mice in tasks that require focused attention but have a predictable cue onset, whereas both groups are impaired to a similar degree in tasks requiring focused attention over a variable delay. Differences between the groups may reflect the neurodegenerative processes seen with aging in Ts65Dn mice that impair brain circuits underlying attention.

Effects of aging in 2N mice

The present study revealed impairments in both learning and attention in 2N mice with advancing age, supporting a growing literature suggesting that aging impairs attention in laboratory rodents (Jones et al. 1995; McGaughy and Sarter 1995; Muir et al. 1999) and humans (Berardi et al. 2001; Greenwood et al. 1997; Parasuraman and Giambra 1991). An effect of aging was seen in the rate at which the 2N mice learned the initial visual discrimination task. 2N-Y mice reached criterion significantly faster than their 2N-O counterparts. This finding is consistent with a report

showing that 3-month-old rats were faster at learning the initial rules of a 5-choice serial reaction time task than 15-month-old rats (Jones et al. 1995). 2N-O mice also performed worse than their younger counterparts in attention task 1, when the cue duration was relatively brief (2-s), but still with a predictable onset time. This performance deficit reflected an increased percentage of inaccurate responses by 2N-O mice; because task rules were well learned by this point, this increased incidence of inaccurate responses is indicative of a greater incidence of missing the cue. Additionally, in attention task 3, 2N-O mice made a significantly higher percentage of inaccurate responses than their younger counterparts, also indicative of an impaired ability to maintain attentional focus.

Morphometric assessment of BFCNs

Cholinergic neurons within the NBM/SI project to the neocortex (Mesulam et al. 1983; Rye et al. 1984) and are important in modulating attention (Hasselmo and Sarter 2011), whereas MS/VDB cholinergic neurons project to the hippocampus (Mesulam et al. 1983; Rye et al. 1984; Sofroniew et al. 1990) and are important in modulating spatial cognition and memory (Hasselmo and Sarter 2011). Prior research concerning BFCNs and Ts65Dn mice focused on MSN neurons, mainly in older Ts65Dn mice (Cooper et al. 2001; Granholm et al. 2000; Holtzman et al. 1996), with virtually no information on the NBM/SI cholinergic perikarya (but see Kelley et al. 2014a, b).

Unbiased stereological assessments of ChAT-ir neurons within the NBM/SI revealed an age-related reduction in the number of these cells in 2N mice, but surprisingly not in Ts65Dn mice. Density of these neurons was not affected by aging in either genotype. However, we found a significant genotype effect for density of these neurons, whereby trisomic mice exhibited a significant reduction in neuronal density relative to 2N controls, which may contribute to their attentional dysfunction. Since there was no effect of age on NBM/SI cholinergic neuron morphology in the trisomic mice, it seems likely that other neurobiological changes underlie the progressive decline in attentional function in these mice. The 2N, but not Ts65Dn mice, exhibited a significant reduction in ChAT-ir neuron volume as a function of age. As a result, cholinergic NBM/SI neurons were significantly larger in Ts65Dn-O compared to 2N-O mice. Humans with asymptomatic AD, who exhibit normal cognition exhibit larger neuronal cell bodies relative to age-matched controls (Iacono et al. 2008). We postulate that the larger size of BFCNs in the aging Ts65Dn brain may reflect a compensatory mechanism in response to the progressive cholinergic signaling deficits (Salehi et al. 2006; Contestabile et al. 2006; Kelley et al. 2016).

Our analyses revealed substantive effects of aging on the morphology of ChAT-ir MS/VDB neurons, some of which varied by genotype. The number of these neurons was significantly reduced with age, to a comparable degree in the two genotypes. ChAT-ir MS/VDB neuron density was significantly reduced by aging, as well as a trend towards reduced density in Ts65Dn mice relative to 2N. This latter finding is consistent with prior reports showing reduced density of MSN neurons in Ts65Dn mice (Ash et al. 2014; Granholm et al. 2000). Volume of ChAT-ir MS/VDB neurons was significantly reduced with aging in 2N but not trisomic mice. As a result, Ts65Dn-O mice exhibited larger cholinergic MS/VDB than 2N-O mice, as observed in the NBM/SI. Prior studies of MS/VDB cholinergic neuron volume have either reported a decrease in Ts65Dn mice relative to 2N mice (Cooper et al. 2001; Granholm et al. 2000; Holtzman et al. 1996; Lockrow et al. 2009, 2011; Salehi et al. 2006) or no difference between groups (Granholm et al. 2002; Seo and Isacson 2005). Although the basis of these inconsistent findings is unknown, one factor that may play a role is the specific marker utilized to label BFCNs. ChAT is present in virtually every BFCN, whereas not all BFCNs contain the cognate nerve growth factor (NGF) receptor TrkA and/or the pan-neurotrophin receptor $p75^{NTR}$ (Mufson et al. 1989; Granholm et al. 2000).

Since attentional function and BFCN morphology were assessed in all mice, the present study allowed for the unique opportunity to investigate functional relationships between these two outcomes. Several significant relationships were observed in the older cohort of mice. Specifically, for the older mice, with genotypes combined, correlational analyses revealed a significant positive relationship between performance on attention task 1 and ChAT-ir cell density within both the NBM/SI and the MS/VDB (Fig. 7). The absence of this correlation in attention task 2 likely reflects the relatively small sample size in the present study. These observed correlations between density of BFCNs and attentional function suggests that density of BFCNs may play a role in attentional function. Interestingly, correlations were not observed between task performance and neuron counts. Moreover, despite differences in density, BFCN counts did not differ significantly between genotypes. This suggests that the neurodegenerative process in Ts65Dn mice may not simply reflect neuron atrophy and loss, but may instead reflect a loss of connectivity resulting from a decrease in neuron density. We also observed a significant negative correlation between performance of the older mice on attention task 2 and ChAT-ir cell volume within the NBM/SI. However, this effect appears to be driven by genotype differences in both neuronal size and attentional performance, i.e., the trisomic mice exhibited poorer task performance and larger NBM/SI neurons.

A technical issue may explain why these correlations were observed only in the older mice. Specifically, four months elapsed between the start of testing and the time of killing, which likely complicates the detection of these relationships in general, but perhaps to a greater extent for the younger mice. Testing on attention task 1 in the younger mice likely began before the start of BFCN neurodegeneration in Ts65Dn mice. However, by the time of sacrifice, this neurodegeneration would be evident, creating a mismatch between the behavioral and neural measures. This lag between the early testing and the postmortem analyses would likely have created less of a mismatch for the older animals, since neurodegeneration was already ongoing in the older Ts65Dn mice at the start of testing. Even though further neurodegenerative changes would likely have occurred in these older animals between the time of testing and killing, it is likely that the relative differences (between individuals) in the degree and rate of degeneration would be maintained, and contribute to (rather than obscure) functional relationships.

Conclusions

The present study revealed that focused and sustained attention are impaired in young Ts65Dn mice, similar to the type of attentional deficits reported for young individuals with DS (Brown et al. 2003; Cornish et al. 2007; Clark and Wilson 2003), providing further support for the validity of the Ts65Dn mouse as a model for DS cognitive dysfunction throughout the lifespan. The observation of attentional dysfunction in the aged trisomic mice replicates previous findings from our lab (Driscoll et al. 2004; Moon et al. 2010), and is consistent with findings in humans with DS after the onset of AD-like neuropathology (Della Sala et al. 1992; Foster 2001; Krinsky-McHale et al. 2008; Levinoff et al. 2004; Pignatti et al. 2005). A goal of this study was to determine whether attentional dysfunction becomes more pronounced with age in Ts65Dn mice. Results indicate that in tasks requiring focused attention but with a predictable cue onset time, Ts65Dn-O mice were more impaired than Ts65Dn-Y mice, but that in tasks requiring attention over a variable pre-cue delay, the two age groups were impaired to a similar degree.

The current investigation also shed new light on the neural substrates of the attentional dysfunction seen in Ts65Dn mice. Attentional deficits in Ts65Dn mice have been posited to be due to a reduced number or atrophy of BFCNs in the NBM/SI. However, the present study did not reveal fewer ChAT-ir neurons within the NBM/SI of the trisomic mice at either age, and neuronal volume was larger for Ts65Dn-O mice than 2N-O mice. In contrast, the density of these neurons was lower in the trisomic mice

than 2N controls for both age groups, which may contribute to the attentional dysfunction seen at both ages. In fact, significant correlations were observed between performance in attention task 1 and density of BFCNs, supporting this hypothesis. Finally, the decreased density of cholinergic NBM/SI neurons seen in trisomic mice did not become more pronounced with aging, suggesting that their age-related decline in attention is related to other neurobiological factors, such as cellular or synaptic dysfunction within the cholinergic projection sites, defects in cholinergic muscarinic or nicotinic receptor subtypes, and NGF transport defects (Salehi et al. 2006). Collectively, these findings revealed that attentional dysfunction in a well-established mouse model of DS and AD is not due to a loss of cholinergic neurons in the NBM/SI, but may reflect, at least in part, reduced density of these neurons and/or abnormal functioning of these neurons due to compensatory changes. A caveat is that although the current study focused on effects in cholinergic neurons, other transmitter-identified systems, including glutamatergic, noradrenergic, and GABAergic are likely to have a role in deficits associated with DS as well (Colas et al. 2013; Faizi et al. 2011; Kaur et al. 2014; Rueda et al. 2012).

Finally, the results provide important information regarding attentional dysfunction in normal rodents during aging. Previous studies have shown that with age, attentional function declines, possibly due to decreased cholinergic function (Jones et al. 1995; McGaughy and Sarter 1995; Muir et al. 1999). The present study corroborated age-related attentional dysfunction in 2N mice, and revealed that both number and volume of BFCNs in the NBM/SI exhibit a corresponding decline. Importantly, the present study demonstrated significant correlations between attention and NBM/SI morphometric indices within the same animals, supporting the importance of this system in attention, as well as aging-related attentional decline.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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