Adaptation of the Arizona Cognitive Task Battery for use with the Ts65Dn Mouse Model of Down Syndrome

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Abstract

We propose and validate a clear strategy to efficiently and comprehensively characterize neurobehavioral deficits in the Ts65Dn mouse model of Down Syndrome. This novel approach uses neurocognitive theory to design and select behavioral tasks that test specific hypotheses concerning what is known from human patients with Down Syndrome. In this manuscript we model in mice the Arizona Cognitive Task Battery used to study human populations with Down Syndrome. This approach extends the utility of mouse models of Down Syndrome by integrating the expertise of clinical neurology and cognitive neuroscience into the mouse behavioral laboratory. Further, by directly emphasizing the reciprocal translation of research between human disease states and the associated mouse models, we demonstrate that it is possible for both groups to mutually inform each other’s research to more efficiently generate hypotheses and elucidate treatment strategies.

Introduction

In recent years, there has been a virtual explosion of understanding regarding the cognitive phenotype of Down Syndrome. The IQ in Down Syndrome is typically at a moderately to severely intellectually disabled range (*i.e.*, FSIQ 25-55) and mental age rarely moves beyond 8 years1. Paradoxically, it has been suggested that early on, Down Syndrome only presented with a mild to moderate intellectual disability (*i.e.*, FSIQ 55-70), but with age the IQ drops as mental age no longer increases with chronological age2. Intriguingly, early motor markers like rolling and sitting up have been shown to be only very subtly slowed in Down Syndrome, but crawling and walking has been shown to be more dramatically delayed. Motor skill development shows the same developmental delays as these early markers of motor abilities2-4.

In the memory domain, Down Syndrome results in deficits for digit or word span as well as general memory deficits with long delays prior to recall5. Working memory, specifically verbal working memory, is disrupted in Down Syndrome. For visual and spatial memory, it appears that Down Syndrome results in specific memory deficits when memory span is increased6. Down Syndrome also results in long term memory deficits, but implicit memory and perceptual priming are normal. This suggests that when memory requires temporal or spatial processes, there is a deficit4,5,7. These data implicate disruptions to hippocampus and medial temporal lobe function in Down Syndrome phenotypes, as well as the prefrontal cortex functional deficits for working memory impairments5,7. Implicit memory, dependent upon different brain areas such as the parietal lobe, appears to be spared, if not slightly facilitated in children and adolescents with Down Syndrome5,7.

To design a battery of behavioral tasks to reliably evaluate cognitive function individuals with Down Syndrome, Edgin and colleagues1,8,9 developed the Arizona Cognitive Task Battery (ACTB). What makes this battery different than other task batteries is that the ACTB has been developed to prevent ceiling and floor effects that far too often confound task performance in pediatric research and to maximize the sensitivity of the ACTB to identify effects that are present in Down Syndrome.

To date, the behavioral assays used to test mouse models of Down Syndrome by and large focus on spatial memory. More specifically, a focus has been placed on the Morris Water Maze test of spatial memory8,10. Later experiments have focused on novel object recognition at short and long delays as a proxy for general memory deficits observed across wide range of mouse disease models11. As a measure of executive function or rostral cortical function, spontaneous alternation has been used12. The majority of motor tests use the rotarod or locomotor behavior in an open field as the primary measure, however more recently the vestibulo-ocular reflex has been used to evaluate cerebellar function in the Ts65Dn mouse13.

Perhaps the most important consideration made in testing mouse models of genetic disorders is that of affect (*i.e.*, emotional salience and/or valence). In other words, negative reinforcement used to motivate performance on the Morris water maze may confound task performance if a given model has anxiety, anhedonia, or depression-like behaviors14,15. To specifically overcome these confounds in a mouse variant of the ACTB (mACTB), we focused on tasks that emphasize spontaneous exploration in mice (*cf.*, Table 1). Particularly, focusing on the tendency of mice to show heightened exploration of changes to their environment. For experiments wherein it was necessary to provide motivation, the mouse was given sucrose pellet rewards that were shown in preliminary testing to be highly palatable. Tests for quality of life and adaptive function were developed for the mouse model, providing another dimension to animal model research not previously widely available to the behavioral researcher.

Results

The week prior to testing, all animals were handled in 15 min daily sessions and given an opportunity to habituate to the clear or red apparatus for at least 15 min and acclimate to sucrose pellet rewards. Behavioral tasks emphasizing exploratory behaviors (under *Hippocampus and Medial Temporal Lobe* in Table 1) were presented in a pseudorandomized order between mice (randomized within the Ts65Dn mice and a 2N wildtype littermate was yoked to a given Ts65Dn mouse to account for any potential task order effects), followed by spontaneous alternation and motor tasks, then response and reversal learning tasks. After these tasks, mice received training on the cheeseboard, and then finally were presented with test designed to evaluate quality of life/adaptive functional measures to reduce the influence of any anxiety measures on later task performance.

To specifically isolate the contribution of spatial and nonspatial cues to task performance, behavioral tasks were run two times, once in a clear box and many extra maze cues, and a second time in a red box without extra maze cues. The rationale for this procedure comes from work reported by Smith et al.16 in Ts65Dn mice and Edgin et al.9 in children with Down Syndrome showing that context is particularly influential during object recognition tasks in children with Down Syndrome relative to typically developing children.

Spatiotemporal Processing

Spatial Attribute

Spatial Navigation

To evaluate spatial navigation and general spatial memory, mice were tested on a dry land version of the Morris water maze (cheeseboard17). The Ts65Dn mice showed deficits relative to 2N control mice for raw latency to find reward (Figure 1; groups (F(1,76)=185.645, p(FDR)<2e-16), no interaction among group and trial block (F(1,76)=0.333, p(FDR)=.566)). These deficits are present as well when the data are adjusted for total latency on trial 1 (groups(F(1,76)=48.44, p(FDR)=1.05e-9)) Ts65Dn mice have impaired learning in the Ts65Dn mice in the adjusted data (F(1,76)=14.74, p(FDR)=.000253). The same pattern of effects was observed for the data when evaluated for raw distance covered to find reward (groups (F(1,76)=88.406, p(FDR)=2.27e-14)) no interaction among group and block (F(1,76)=0.258, p(FDR)=.613). Similarly to the latency data, an interaction emerges with Ts65Dn mice showing a shallower learning curve when the data are adjusted for total distance on trial 1 (groups (F(1,76)=25.194, p(FDR)=3.32e-6), interaction (F(1,76)=3.887, p(FDR)=.0523)).

During the probe trial, Ts65Dn mice spent significantly less time in the quadrant where the reward was previously located (F(1,18)=91.25, p(FDR)=1.8e-6). Ts65Dn mice also on average were located a further distance away from the previously rewarded spatial location (F(1,18)=41.7, p(FDR)=4.47e-6).

Spatial Processing

To evaluate dentate gyrus dependent spatial processing, mice were tested for detection of a metric change (Figure 2; and16), Ts65Dn mice showed significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=39.38, p(FDR)=6.44e-6) as well as the red box (F(1,18)=29.94, p(FDR)=3.38e-5). Deficits in both the clear and red box suggest that metric processing is specifically impaired in Ts65Dn mice.

To evaluate parietal lobe dependent spatial processing, mice were tested for detection of a topological change (Figure 2), Ts65Dn mice showed significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=78.52, p(FDR)=5.55e-8) but not for the red box (F(1,18)=1.489, p(FDR)=.238). Deficits in only the clear box suggests that topological processing is only impaired when extra-maze cues are present, suggesting a general spatial memory deficit rather than one specific to topological processing.

To test general spatial memory, mice were tested for detection of a change in the spatial location of a visual object (Figure 2), Ts65Dn mice showed significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=36.39, p(FDR)=1.05e-5) as well as in the red box (F(1,18)=62.0, p(FDR)=3.07e-7), suggesting spatial novelty detection deficits in Ts65Dn mice.

Temporal Attribute

To test CA1 function in Ts65Dn mice, mice were tested for a simple temporal ordering task (Figure 2; and18). Ts65Dn mice did not show significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=68.24, p(FDR)=1.55e-7) but not for the red box (F(1,18)=2.267, p(FDR)=.149). These data suggest that the presence of spatial cues, but not temporal ordering resulted in deficits in the clear box. For the novelty detection task run as a control for temporal ordering, Ts65Dn mice did not show significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=82.78, p(FDR)=3.74e-8) but not for the red box (F(1,18)=2.909, p(FDR)=.105). These data suggest that the presence of spatial cues, but not temporal ordering or novelty detection resulted in deficits in the clear box.

Sensory/Perceptual Attribute

To test perirhinal function in Ts65Dn mice, a configural feature ambiguity test was given (Figure 3; and16). Ts65Dn mice did not show significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=34.13, p(FDR)=1.56e-5) but not for the red box (F(1,18)=.021, p(FDR)=.984). These data suggest that the presence of spatial cues, but not configural feature ambiguity resulted in deficits in the clear box. Ts65Dn mice were not impaired in a configural ambiguity control task. There was a main effect for groups for the clear box (F(1,18)=12.27, p(FDR)=.00254) but not for the red box (F(1,18)=.012, p(FDR)=.916). These data suggest that the presence of spatial cues, but not configural feature novelty detection ordering resulted in deficits in the clear box.

Object memory was tested in Ts65Dn mice using object recognition memory at 1 and 24 hours (Figure 3), Ts65Dn mice did not show significant impairments relative to 2N control mice. There was a main effect for groups for the clear box (F(1,18)=29.51, p(FDR)=3.68e-5) but not for the red box (F(1,18)=.908, p(FDR)=.353). These data suggest that the presence of spatial cues, but not object recognition resulted in deficits in the clear box. For object recognition memory at 24 hours, there was a main effect for groups for the clear box (F(1,18)=46.23, p(FDR)=2.29e-6) as well as for the red box (F(1,18)=31.36, p(FDR)=2.58e-5). These data suggest that at 24 hours, the Ts65Dn mice were unable to retrieve the memory for the object, whereas they were able to do so at 1 hour.

Rule Based Memory System/Executive Function

Spontaneous alternation was used to test working memory in the Ts65Dn mice (Figure 4), Ts65Dn mice showed fewer alternations than 2N control mice (F(1,18)=23.85, p(FDR)=.00012).

To evaluate inhibitory control and the ability to learn a turn response (Figure 4), Ts65Dn mice took significantly longer to learn the rule than 2N control mice. There was a main effect for groups (F(1,76)=4.24, p(FDR)=.013) and a main effect for block of trials (F(1,76)=502.86, p(FDR)<2e-16). There was also an interaction among group and block (F(1,76)=7.82, p(FDR)=.00654). This interaction was the result of the Ts65Dn mice taking longer to learn the rule. For the final block of 20 trials, there were no differences in performance for Ts65Dn and 2N control mice.

To evaluate rule reversal learning (behavioral inflexibility) in Ts65Dn mice, the reversal of a turn response was evaluated (Figure 4). Ts65Dn mice took significantly longer to learn the rule than 2N control mice. There was a main effect for groups (F(1,76)=4.952, p(FDR)=.029), a main effect for block of trials (F(1,76)=24.62, p(FDR)=4e-6). There was also a trend toward there being an interaction among group and block (F(1,76)=3.21, p(FDR)=.077). This nonsignificant interaction was the result of the Ts65Dn mice taking longer to learn to reverse the rule. In fact, the Ts65Dn mice were only impaired relative to the 2N control mice for the first block of 20 trials. For the remaining blocks of trials there were no differences in performance for Ts65Dn and 2N control mice. There was also a main effect for groups for the trial at which the mice changed preference from old rule to new rule (changepoint; F(1,18)=21.43, p(FDR)=.000208)). For the first 20 trials of reversal learning, Ts65Dn mice showed a greater number of perseverative errors (F(1,18)=11.98, p(FDR)=.00278). For trials 21-40, there was no difference between Ts65Dn mice and 2N control mice for regressive errors (F(1,18)=.287, p(FDR)=.599).

Motor Function

For the capellini task of manual dexterity (Figure 5; and19), Ts65Dn mice showed significant impairments relative to 2N control mice. There was a main effect for latency, with Ts65Dn mice taking longer to eat the pasta on average (F(1,18)=14.74, p(FDR)=.0012). Ts65Dn mice also made a greater number of pasta handling errors (F(1,18)=92.68, p(FDR)=1.6e-8). There was also a main effect for groups for the number of times the paws came together (F(1,18)=42.34, p(FDR)=4.06e-6), for the number of times the mouse lost contact with the pasta (F(1,18)=20.35, p(FDR)=.00027), and the number of times the mouse pulled the pasta with their mouth rather than using the hands to move it (F(1,18)=21.46, p(FDR)=.000207).

During a parallel rung walking task (Figure 5; and20), Ts65Dn mice showed significant impairments relative to 2N control mice. There was a main effect for the number of foot slips in a 1 minute session (F(1,18)=27,32, p(FDR)=5.7e-5). When adjusted for number of steps, Ts65Dn mice still showed a greater number of foot slip errors (F(1,18)=11.70, p(FDR)=.00305).

Adaptive Function/Quality of Life

Ts65Dn mice showed significant impairments relative to 2N control mice for measures of nesting (Figure 6). Ts65Dn mice took longer to make contact with the nesting material (F(1,18)=152.9, p(FDR)=3.1e-10), for the time it took for them to dig in the media (measured from time of first contact) (F(1,18)=318.6, p(FDR)=6.79e-13), and the time it took from starting to dig to finish the nest (F(1,18)=94.3, p(FDR)=1.4e-8).

Ts65Dn mice showed significant impairments relative to 2N control mice for neophobia (Figure 27). Ts65Dn mice took longer to eat a novel food in a familiar environment (F(1,18)=19.59, p(FDR)=.000326), took longer to eat a familiar food in a novel environment (F(1,18)=40.87, p(FDR)=5.09e-6), and took longer to eat a novel food in a novel environment (F(1,18)=83.74, p(FDR)=3.44e-8).

Grouping Tasks by Domain: Principal Component Analysis

To determine if there were a general structure to the tasks being used in the mouse ACTB, a principal component analysis was performed. This PCA analysis identified 2 main clusters, with one axis accounting for 57.76% of the variance, and the other only 9%. This clustered the tasks into groups with all the spatiotemporal tasks and spontaneous alternation in one group, and the executive, motor, and adaptive function tasks comprising a second cluster. Although it seems odd for the alternation to be sorted in with MTL dependent tasks, this makes sense given the spontaneous alternation protocol was also an unrewarded delayed alternation task, known to be hippocampus dependent. When this clustering was used to sort the mice into groups, the principal axis was sufficient to sort the mice into appropriate Ts65Dn and 2N control groups, suggesting primary MTL and rostral cortical dysfunction in Ts65Dn mice, thus phenocopying reports in human Down Syndrome8.

Discussion

General Discussion

Overall, these data clearly demonstrate that the Ts65Dn mouse do in fact show a similar pattern of behavioral deficits as individuals with Down Syndrome on the mACTB. Ts65Dn mice show clear deficits for spatial processing tasks dependent upon the dentate gyrus with sparing of temporal processing dependent upon the CA1 subregion (*cf.,*21,22). Similarly, it appears that spatial processing dependent on neocortical processing is spared18. There are also specific deficits for motor function, executive function, and adaptive function. These deficits phenocopy the results from the ACTB used in testing children with Down Syndrome1,8.

These data support earlier theories that suggested there were specific deficits to spatial memory in Down Syndrome. What these data clarify are the neural substrates and specific domains of MTL function are impaired in Down Syndrome. There are specific deficits on tasks that test dentate gyrus function, but sparing of function on tasks that test perirhinal and CA1 function. Similarly, there are specific deficits in the Ts65Dn mouse that are attributable to cerebellar function and executive functional deficits attributable to the rostral cortices (human prefrontal cortex).

An important consideration in adopting a behavioral screen like this mACTB is the relative throughput for the tasks. All of the tasks used to test MTL function take 30 minutes per session of testing, and can be repeated numerous times on any given mouse after 24 hours post the first test. The motor and adaptive function tests are similarly high throughput, as is the spontaneous alternation task. The only tasks that require a significant time investment are the dry land watermaze on the cheeseboard and the rule acquisition and reversal learning tasks. The cheeseboard follows a standard water maze protocol that lasts 5 days, and the response learning and reversal learning tasks together take an additional week. A second consideration is adopting the mACTB is the advantage of the anatomical specificity of known neural substrates underlying each behavioral task. As such, these tasks can be used to dissociate function of brain areas within the mouse models being tested. The final consideration is the lack of negative reinforcement or aversive stimulus. This means mouse models displaying depression, anxiety, or anhedonia are testable using the mACTB.

That these deficits in the mouse and human are comparable suggests that the mACTB may be useful for guiding the development of treatment strategies by providing reliable, valid behavioral endpoints and outcome measures (*cf.,*15,18). Similarly, the mouse mACTB appears to be a useful tool for studying behavioral prodrome of early Alzheimer-like pathology and cognitive decline in mouse models related to Down Syndrome.

Online Methods

Animals

In this study, 10 segmentally trisomic Ts(1716)65Dn male mice and 10 wild type littermates were obtained from Jackson Laboratories (Bar Harbor, ME) and tested at 5-7 months of age, at an average weight of 33 +/- 3.8g (standard error). Animals were kept on a 12-h light/dark cycle, in a temperature and humidity controlled environment with *ad libitum* access to food and water. All behavioral tests were conducted during the light portion of the cycle (06:00-18:00). Mice were housed in same-genotype groups of 2-3 per cage. Animal care and experimental testing procedures conformed to NIH, IACUC, and AALAC standards and protocols.

The week prior to testing, all animals were handled in 15 min daily sessions and given an opportunity to habituate to a clear or red box 40x40x40 cm for at least 15 min and acclimate to sucrose pellet rewards. Behavioral tasks emphasizing exploratory behaviors (under *Hippocampus and Medial Temporal Lobe* in Table 1) were presented in a pseudorandomized order between mice (randomized within the Ts65Dn mice and a 2N wildtype littermate was yoked to a given Ts65Dn mouse to account for any potential task order effects; *cf.*,18), followed by spontaneous alternation and motor tasks, then response and reversal learning tasks. After these tasks, mice received training on the cheeseboard, and then finally were presented with test designed to evaluate quality of life/adaptive functional measures to reduce the influence of any anxiety measures on later task performance. Full task protocols and descriptions are given in the *Supplemental Methods*.

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Table 1

| **Brain Regions/Test** | **Domain/Ability Assessed** | **Analogous Task in Mice** |
| --- | --- | --- |
| **Benchmark** |  |  |
| KBIT-II verbal subscale | Receptive and Productive language | *not modeled* |
| KBIT-II nonverbal subscale | Problem solving | *not modeled* |
| Scales of Independent behavior-revised | Adaptive function | Nesting, Neophobia |
| CANTAB spatial span | Immediate memory for spatiotemporal sequences | *not modeled* |
| **Prefrontal Cortex** |  |  |
|  | ***Executive Function*** |  |
| Modified dots task | Inhibitory control and working memory | Spontaneous Alternation |
| CANTAB IED | Set Shifting | Rule response learning, Rule reversal learning |
| **Hippocampal and Medial Temporal Lobe** |  |  |
|  | ***Spatial Attribute*** |  |
| CANTAB PALS | Spatial associative memory | Location Recognition |
| Virtual Water Maze | Spatial memory/navigation | Cheeseboard |
| *not evaluated* | Spatial relationships | Metric, Topological |
|  | ***Temporal Attribute*** |  |
| *not evaluated* | Temporal processing/Sequence learning | Temporal order for visual objects |
|  | *Sensory/Perceptual Attribute* |  |
| *not evaluated* | Object recognition | Configural feature ambiguity, Object recognition |
| **Cerebellum** |  |  |
|  | ***Motor Function*** |  |
| Finger Sequencing Task | Motor sequencing | Capellini handling |
| NEPSY visuomotor precision | Visuomotor tracking/Hand-eye coordination | Parallel rung walk |
| CANTAB SRT | Motor response time/Attention | *not modeled* |

Table 1. Mouse Model for the Arizona Cognitive Task Battery (mACTB). In order to isolate the contribution of of spatial features from sensory/perceptual features on task performance, all paradigms in the Hippocampus and Medial Temporal Lobe section were run twice, once in the presence and once absence of environmental cues. Note that within the Hippocampus and Medial Temporal Lobe section there are additional tasks run in the mouse model that do not appear in the ACTB that are intended to more fully characterize any deficits observed in the mouse model and potentially inform research into human Down Syndrome.

Figure Captions:

Figure 1: Cheeseboard Performance. a. Raw latency (s) to obtain reward. b. Percent latency to obtain reward each day scaled by latency on Day 1. c. Raw distance (cm) to obtain reward. d. Percent distance to obtain reward each day scaled by latency on Day 1. e. probe trial performance: time in target quadrant (25% is chance) f. probe trial performance: average distance from target hole (in cm).

Figure 2: Spatiotemporal Processing. a. Metric/Coordinate processing performance in the presence and absence of extra-maze, distal cues. b. Topological/Categorical processing performance. c. Temporal Ordering for visual objects performance. d. Novelty Detection performance. e. Spatial Location recognition. All tasks run twice, once in the presence of extramaze cues and once in a red box blocking all extramaze cues.

Figure 3: Sensory/Perceptual Processing. a. Visual object recognition task at 1 hour delay the presence and absence of extra-maze, distal cues. b. Visual object recognition task at 24 hour delay the presence and absence of extra-maze, distal cues. c. Configural feature ambiguity test in the presence and absence of extra-maze, distal cues. d. Novel Configural ambiguity control test in the presence and absence of extra-maze, distal cues. All tasks run twice, once in the presence of extramaze cues and once in a red box blocking all extramaze cues.

Figure 4: Executive Function. a. Spontaneous/Delayed Alternation performance. b. Response rule reversal raw data converted to cumulative response. c. Reversal Learning. d. Changepoint, or trial at which each mouse learned to reverse the rule. e. Number of Perseverative errors in trial 1-20 trials. f. Number of Regressive errors in trials 20-40.

Figure 5: Motor Function. a. Latency to consume capellini. b. Total number of atypical behaviors. c. Number of times mouse paws came together. d. Number of times mouse lost contact with capellini with one paw. e. Number of times mouse pulled capellini with mouth rather than paw. f. Raw number of foot slips in a 1 minute period of free exploration. g Number of foot slips scaled by the total number of steps.

Figure 6: Adaptive Function. a. Latency to contact nesting material. b. Latency to first dig in nesting material. c. Latency to complete nest. d. Latency to start to consume novel food in a familiar environment. e. Latency to start to consume familiar food in a novel environment. f. Latency to start to consume a novel food in a novel environment.

Supplemental Methods

Tests of Spatiotemporal Attributes

As part of testing spatiotemporal attributes in the Ts65Dn mice, Smith et al.16 demonstrated that it is important to consider a role for spatial context interfering with tests of sensory/perceptual or temporal processing (*e.g.*, object recognition or sequence learning tasks). This is due to specific alterations in plasticity that have been demonstrated in the dentate gyrus of the Ts65Dn mice and abnormal anatomy of the dentate gyrus of the hippocampus in individuals with Down Syndrome. To eliminate confounding effects of contextual/spatial memory deficits in Ts65Dn mice, we performed all spatiotemporal experiments twice, once in a red box and no distal cues present, and once with a transparent box and a number of distinct distal cues available.

Spatial Navigation

Cheeseboard

Method: Each mouse was habituated to the cheeseboard for 30 min the day prior to experimentation with food pellets distributed in each hole. At the beginning of each trial, a sucrose reward pellet was placed in one of the holes of the cheeseboard (located within the midpoint of the NorthEast, NorthWest, SouthEast or SouthWest quadrant). A mouse was then released at one of the cardinal points (*e.g.,* North, South, East, or West at the edge of the cheeseboard) as latency in seconds and distance in centimeters travelled to locate and consume the reward was recorded. Each day, the mouse received a trial from each of the four cardinal directions (order randomized between mice and between days within mice). There were 5 minutes separating each trial for each mouse. After the fourth day of training, the mice were given a probe trial wherein there was no reward. The search patterns of the mice were evaluated.

Spatial Processing

Metric / Coordinate Processing

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the metric/coordinate test, two objects were placed in the box separated by 25 cm (from inner edges) and mice were allowed to explore the objects for 15 minutes. After a 5 min interval during which the mice were covered by a heavy cup, the objects were moved closer together to an 8 cm separation and the mouse was allowed to explore for 5 min. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. A ratio value approaching 1 suggest the mouse dramatically explored the change.

Topological / Categorical Processing

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the topological/categorical test, four objects were placed in a square in the box separated by 25 cm (from inner edges) and mice were allowed to explore the objects for 15 minutes. After a 5 min interval during which the mice were covered by a heavy cup, the front two objects were transposed, and the mouse was allowed to explore for 5 min. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. A ratio value approaching 1 suggest the mouse dramatically explored the change.

Location Recognition

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the location recognition test, two objects were placed in the box separated by 25 cm (from inner edges) and mice were allowed to explore the objects for 15 minutes. After a 5 min interval during which the mice were covered by a heavy cup, one of the objects was moved at a diagonal to a new location (still 25 cm separation between the two objects), and the mouse was allowed to explore for 5 min. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. A ratio value approaching 1 suggest the mouse dramatically explored the change.

Temporal Attribute

Temporal Ordering for Visual Objects

Method: During session 1, two identical copies of a first object (object 1) were placed at the ends of the box 2.5 cm from the end walls and centered between the long walls. The mouse was placed in the center of the box facing away from both objects. The mouse was given 5 min to freely explore the objects. After 5 min, the mouse was removed to a small holding cup for 5 min. During this time, the first objects were replaced with two duplicates of a second object (Object 2). For Session 2, the mouse was again placed in the apparatus and allowed to explore. After 5 min, the mouse was removed to the holding cup for 5 min and the objects were replaced with two duplicates of a third object (Object 3). For Session 3, the mouse was given 5 min to explore. After 5 min, the mouse was removed into a small cup for 5 min and an unused copy of the first and an unused copy of the third object were placed into the box. The mouse was again placed into the box and allowed to explore the two objects (*i.e.*, Objects 1 and 3) during a 5 min test session. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration of each object during the test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing an absolute preference for the third over the first object. A ratio value approaching 1 suggest the mouse strongly explored the first over the third object.

Temporal Order Control: Novelty Detection for Visual Objects

Method: In addition to reflecting impaired temporal ordering, increased exploration of the first object over the third could also be interpreted as being due to difficulty in remembering the first object prior to the test session. To minimize and control for such general memory deficits, a novelty detection of visual objects task was performed. Briefly, on a different day mice received three sessions during which they were allowed to explore three novel sets of objects (Objects 4, 5, 6) similarly to the temporal ordering tasks. During the test session, the first object and a novel fourth object (Object 7) were presented and the mice were allowed 5 min to explore. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration of each object during the test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing an absolute preference for the familiar over the novel object. A ratio value approaching 1 suggest the mouse strongly explored the novel over the familiar object.

Sensory/Perceptual Attributes

Feature Ambiguity

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the configural recognition condition, mice were placed for 15 min in the red box containing two compound objects, AB and CD, separated by 15 cm. Following a 5 min delay under a heavy cup, the mouse underwent a 5-min Test Phase in which one object from the Study Phase remained the same (AB) and the other compound object is created from one component of each of the previous familiar objects, (*e.g.*, AD). That is, the “novel” object (AD) is composed of the same elements, but rearranged into a novel configuration. Therefore, the object is “novel” by virtue of its configuration, not by its elements, each of which was present in one of the original compound stimuli. Exploration of each compound object was scored as a single unit. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. A ratio value approaching 1 suggest the mouse dramatically explored the change.

Feature Ambiguity Control: Novelty Detection for Configural Objects

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the configural recognition condition, mice were placed for 15 min in the red box containing two compound objects, AB and CD, separated by 15 cm. Following a 5 min delay under a heavy cup, the mouse underwent a 5-min control task during which CD was replaced by two never before seen objects (EF) was also performed. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. A ratio value approaching 1 suggest the mouse dramatically explored the change.

Object Recognition at 1 hour and 24 hour delays

Method: Each mouse had previously been habituated to the clear and red experimental boxes. For the object recognition test, two objects were placed in the box separated by 25 cm (from inner edges) and mice were allowed to explore the objects for 15 minutes. After a 5 min interval during which the mice were covered by a heavy cup, one of the objects was replaced by a novel object that had never before been experienced by the mouse, and the mouse was allowed to explore for 5 min. This procedure was carried out in the clear box that allowed the mouse to see the extra-maze, distal cues as well as in the red box that blocked the ability of the mouse to see these cues. This procedure was carried out in each box separately for delays of 1 hour and 24 hours. Exploration during the last 5 min of habituation and during the 5 min test session were converted into a ratio value ranging [-1,1] to control for overall exploration. As such, a ratio value approaching -1 is interpreted as the mouse showing continued habituation and thus not noticing the change. a ratio value approaching 1 suggest the mouse dramatically explored the change.

Tests of Executive Function

Spontaneous Alternation

Method: Mice were placed in the stem of a Y maze and allowed to explore. Whenever the mouse entered one of the arms of the Y maze with all four limbs their response was recorded. Upon reaching the end of the arm, the mouse was gently picked up and replaced in the stem of the Y maze. The number of times the mouse alternated (*i.e.,* did not repeat the previous turn), it was recorded as an alternation.

Response Learning

Method: Mice were placed in the stem of a plus maze with one of the arms blocked off (forming a T maze). Mice were given five trials to determine if there was any preference for one direction over the other. As no such preference was observed, mice were randomly assigned the rule to turn right or turn left. Mice received 20 trials per day for 4 days. Entry into an arm with all four limbs was recorded as a choice and mice were not allowed to self correct when they made mistakes. Upon reaching the end of the arm, the mouse was gently picked up and replaced in the stem of the plus maze.

Reversal Learning

Method: The day after mice finished training on response learning, they received 80 trials of reversal training. This means that the turn the mice had just learned to make for reward was now incorrect, rather the mice had to make the opposite turn to receive reward. Upon reaching the end of the arm, the mouse was gently picked up and replaced in the stem of the plus maze. Number of previously correct choices made were recorded as errors and error type was evaluated as perseverative or regressive. Additionally, a behavioral change point algorithm was used to define the point at which each mouse consistently switched their responses from the previously learned rule to the new rule.

Motor Function

Capellini Handling

Method: Mice were habituated over a weekend to dried capellini pasts in their cages. Each mouse was placed in a 250 mL beaker and given a 5 cm piece of dried capellini. Their behaviors while eating were recorded for an offline analysis of their motor behaviors. Their latency to finish each piece of pasta was recorded, as were abnormal behaviors including the mouse having its paws together while eating, losing contact with the pasta with one or both paws, and using the mouth to pull the pasta rather than using the digits to feed the pasta into the mouth.

Parallel Rung Walking

Method: Mice were placed in a box measuring 15 cm squared with 1.5 mm diameter parallel rungs making up the floor. The mice were allowed to freely explore the box for 5 minutes. The number of times a paw slipped through the parallel rod floor beyond the wrist or ankle, a "foot slip" error was recorded. The total number of steps were also recorded to use as a normalizing factor.

Adaptive Function

Nesting Behaviors

Method: Sawdust was used to fill a 10 cm long piece of 2" (~5 cm) diameter PVC pipe that was capped at one end. This pipe was placed in a cage with each mouse and the latency to contact the sawdust in the pipe, the latency to start digging in the sawdust, and the latency to finalize the nest were recorded.

Neophobia

Method: Mice were given three neophobia tests. The first was in their homecage. Each mouse was provided a food they had never encountered (Cheerios cereal) and the latency to take the first bite was recorded. The second test was each mouse was placed on a large platter in a bright area in the testing room and the latency to take a bite from a reward pellet (familiar food) was recorded. The final test consisted of each mouse being placed in a novel white box and fed a Cheerio that had been stored with thyme overnight, resulting in a novel food. Again, latency to take the first bite was recorded.

Statistical Analysis

Dependent Measures and Data Visualization.

For exploratory tasks, ratio values were computed after the following formula: Exploration of the object of interest (or all objects in the 5 min session of interest) minus the exploration of the other objects or last 5 min of the habituation session. This was divided by the sum of all exploration across both sessions or of both objects. As a formula this is depicted as: (A-B)/(A+B).

For the reversal learning, the number of perseverative errors (continuing old rule) during the first 20 (1-20) trials were computed. The number of regressive errors (returning to old rule) were calculated during trials 21-40. A frequentist change point algorithm developed by Gallistel et al23 and translated in the R programming language by Diep et al.24 was used to compute the point at which each mouse showed evidence for having learned to apply the new rule (algorithm openly available for download at <http://github.com/mrhunsaker/Change_Point>).

Data are all plotted in DataGraph (3.21 beta, Visual Data Tools, Inc. Chapel Hill, NC.). Ratio data and computed factors are plotted as a bar plot to the mean with all collected data points overlain. Repeated data are presented as a line graph at the mean of each block, with all data points overlain.

Tests for equal variance and heteroscedasticity.

Prior to statistical analyses, the data were tested for normalcy (Shapiro–Wilk test) and homoscedacity (Browne–Forsythe test). Repeated measures were evaluated for sphericity using Mauchly's test of sphericity and necessary adjustments were made using the Huhn-Feldt correction using R 3.0.1.

Parametric Statistical Analysis

Once deemed appropriate, further statistical analyses were performed using parametric analyses of variance. For exploratory task ratios and computed factors were compared using a one-way analysis of variance (ANOVA) with groups (2N control, Ts65Dn). For acquisition tasks wherein learning was quantified across trials as well as locomotor data, statistical analyses were performed using a mixed model ANOVA with group (2N control, Ts65Dn) as a between groups factor and block of trials as a repeated within factor. If locomotor activity was significantly different between the groups, locomotor activity was included in the statistical analysis as a covariate.

All results were considered significant at an α < .05 and Power (1-ß) ≥ .8: Analyses were performed to determine observed power and effect size for all reported effects. Statistical analyses were performed in R 3.0.1 language and environment and statistical power was calculated using both R and the statistical program G\*Power 3. All reported p values were adjusted for False Discovery Rate using a custom script written in R 3.0.125.

Principal Component Analyses

PCA were run by applying the functions in the *FactoMineR* package in R 3.0.1 and follow up analyses with functions in the *MClust*, *HClust*, *PVClust*, and *Psych* packages. These models are presented to provide a qualitative description of the experiments tasks and to demonstrate that the behavioral domain evaluated by these tasks parallel the cognitive domains studied using the ACTB in populations with with Down Syndrome.

Supplementary References

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24. Diep, A. A. et al. Female CGG knock-in mice modeling the fragile X premutation are impaired on a skilled forelimb reaching task. *Neurobiol Learn Mem* **97**, 229-234 (2012).

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