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Dentate Gyrus Mediates Cognitive Function in the Ts65Dn/DnJ Mouse Model of Down Syndrome

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Abstract

In the Ts65Dn/DnJ mouse model of Down syndrome (DS), hippocampal deficits of learning and memory are the most robust features supporting this mouse as a valid cognitive model of DS. Although dentate gyrus (DG) dysfunction is suggested by excessive GABAergic inhibition, its role in perturbing DG functions in DS is unknown. We hypothesize that in the Ts65Dn/DnJ mouse, the specific role of the DG is disturbed in its support of contextual and spatial information. Support for this hypothesis comes from rats with DG lesions that show similar deficits. In order to test this hypothesis, we have developed a novel series of spontaneous exploratory tasks that emphasize the importance of recognizing spatial and contextual cues and that involve DG function. The results with this exploratory battery show that Ts65Dn/DnJ mice are impaired in DG-dependent short-term recognition tests involving object recognition with contextual cues, in place recognition and in metric distance recognition relative to wild type littermate controls. Further, whereas Ts65Dn/DnJ mice can recognize object novelty in the absence of contextual cues after a 5-min delay, they cannot do so after a delay of 24 h, suggesting a problem with CA1-mediated consolidation. The results also show that Ts65Dn/DnJ mice are not impaired in tasks (object recognition and configural object recognition) that are mediated by the perirhinal cortex (PRh). These results implicate the DG as a specific therapeutic target and the PRh as a potential therapeutic strength for future research to ameliorate learning and memory in DS.

Keywords

object recognition; spatial location recognition; configural object recognition; hippocampus; developmental disorders

INTRODUCTION

The Ts65Dn/DnJ mouse is a useful model for examining the neuronal mechanisms that may subserve the behavioral and cognitive phenotypes of Down syndrome (DS). This mouse is trisomic for a segment of mouse chromosome 16 (MMU16) that contains many (~67%) of the genes homologous to human chromosome 21 and displays several physical and cognitive

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phenotypes of persons with DS, including memory deficits on tasks that are dependent on hippocampal (HC) function. Seregaza et al. (2006) have provided a review of learning and memory problems of the Ts65Dn mouse. However, recent data have differentiated sub-regional functions for the HC, providing a unique opportunity to genetically parse these and then to target modulations using the Ts65Dn mouse. The dentate gyrus (DG) is central for contextual and spatial integration as well as pattern separation, the CA3 region for pattern completion, the CA1 for temporal ordering of objects and places, and the perirhinal cortex (PRh) for configural object recognition (Kesner et al., 2012).

However, there is little evidence for the importance of DG dysfunction in learning and memory deficits of the Ts65Dn mouse, although this has been speculated from its electrophysiological and neuroanatomical features. Previous Ts65Dn research has shown significant decreases in neuronal number, abnormal synaptic morphology and deficits in long-term potentiation related to excessive GABA inhibition in the DG (Belichenko et al., 2004; Popov et al., 2011; Belichenko et al., 2009; Kleschnikov et al., 2012). In order to examine the role of the DG in learning and memory, it is important to develop tasks that are critically mediated by the DG. We used a new approach to study Ts65Dn/DnJ mice by employing exploratory tasks to access recognition memory without high stress and anxiety. These tasks emphasize novelty recognition driven by rodents' propensity to explore new and unfamiliar stimuli (Ennaceur and Delacour, 1988; Ennaceur, 2010). Tests now include measures of place recognition and metric change, both shown to be sensitive to lesions of HC, and especially DG, in rats (Deese and Kesner, 2013; Goodrich-Hunsaker et al., 2008; Lee et al., 2005). Further distinction of HC and DG functions are based on a task to detect configural object recognition that is dependent on PRh and independent of HC function. It has been shown that performance on this task is spared in rats with DG lesions (Kesner, Ford, and Taylor, in preparation).

In order to assess the capability of Ts65Dn/DnJ mice to integrate contextual information and thereby provide behavioral evidence for localized DG dysfunction, variations of spontaneous novelty detection paradigms were employed that can be performed with or without the presence of contextual cues. The test paradigm used short delays (5 min) within a red box that prevented the use of context and a clear box with stimuli that could be encoded as context. The next two experiments measured spatial and metric recognition memory in red and clear boxes. With a new cohort of Ts65Dn/DnJ mice, the fourth experiment investigated object recognition at long delays (24 h) in the red box. The final experiment, using this new cohort, expanded on the object recognition paradigm to assess short-term recognition memory for configural arrangements.

MATERIALS AND METHODS

Subjects

In this study, trisomic male Ts65Dn/DnJ mice and their age-matched wild type littermates were obtained from Jackson Laboratories (Bar Harbor, ME) and tested at ~3–6 months of age, at an average weight of 25 g. 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 tests were conducted during the light portion of the cycle. All animals were housed

individually to limit the interference of social interaction on test performance. Animal care and experimental testing procedures conformed to NIH, IACUC, and AALAC standards and protocols.

As detailed by Jackson Laboratory, segmentally trisomic Ts(17¹⁶)65Dn mice have three copies of genes at the distal 15% of mouse Chr 16 and span the region orthologous to genes on human Chr 21. These extra genes, centromere, and about 5% of proximal mouse Chr 17 are contained in the small extra chromosome derived from a reciprocal translocation. About 15% of the distal end of Mouse chromosome 16 is fused to the centromeric end of chromosome 17 to form the small translocation chromosome. The translocation breaks mouse Chr 16 just proximal to the amyloid precursor protein (App) gene and contains the Chr 21-homologous genes from App to the telomere. The Ts65Dn/DnJ stock, commercially available from Jackson Laboratory, is homozygous for the wild type allele for retinal degeneration. The stock is maintained by repeated backcrossing of Ts65Dn females to B6EiC3H F1 hybrid males derived from a new congenic strain of C3H mice. This new congenic strain (C3Sn.BLiA-*Pde6b*¹) lacks the blindness causing recessive mutant allele. Thus, all trisomic mice purchased were tested without concern for retinal degeneration.

Experimental Design

Experiments consisted of five exploratory paradigms based on the inherent tendency of rodents to differentially explore novel stimuli over familiar stimuli: short-term object recognition at 5 min (Ennaceur, 2010), place recognition (Langston and Wood, 2010; Lee et al., 2005), metric spatial processing (Goodrich-Hunsaker et al., 2008), long-term object recognition at 24 h (Fernandez et al., 2007), and configural object recognition (Bartko et al., 2007). In each paradigm, there is a Study Phase during which mice display reduced exploration over time as a function of habituation and a Test Phase based on novelty detection that is interpreted to reflect recognition memory. The week prior to testing, all animals were handled in 1–2 min daily sessions and given an opportunity to habituate to the clear or red apparatus for at least 10 min. Each experimental session was separated by a minimum 48-h interval. It is important to note that the animals were started from the same position for every test, allowing them to orient spatially even in the absence of contextual information. The first three testing paradigms (short-term object recognition, place recognition, and metric spatial processing) were conducted in both the clear (contextually rich) and the red (minimal context) apparatus (Fig. 1) and the last two tests (long-term object recognition and configural object recognition) were conducted in the red box only. The order of presentation for these experiments was counterbalanced when possible, although it is important to note that order effects are not normally observed in exploration tasks. Two cohorts of animals were tested on object recognition at 5 min. One of these cohorts was also used for place recognition while the other was used for metric spatial processing. The last two experiments used two new cohorts, one used for object recognition at 24 h and the other for configural object recognition.

Apparatus

The apparatus for these experiments consisted of a large Plexiglas box 40 × 40 cm² with clear walls 40 cm in height and a dark grey floor. An inset made of translucent red Plexiglas

40 × 40 cm² was constructed for easy insertion and removal from the original clear box, therefore enabling the experimenter to block distal cues in the testing environment when desired. The box was placed on a circular white table 1 m in diameter. Four distinct two-dimensional black and white cues were placed 30 cm away from center of each side of the box (see Fig. 1 for a photograph of the testing apparatuses). Exploration was recorded with an overhead video camera and the duration of exploration was measured with a stopwatch. Objects were made from various washable, non-porous materials (plastic, metal, glass, etc.), 2–7 cm in height and had various color, pattern, and texture schemes to ensure each object was visually distinct. To prevent odor cues, the boxes and objects were disinfected and deodorized with a sterilizing cleaning agent after each use. The animal was presented with entirely novel object set for every experiment.

Dependent Measures

For all tasks, the amount of time the animal spent exploring each object was recorded as the dependent variable. Exploration was defined as any investigative behavior where mice have active and direct contact with an object. Such behaviors included orienting head within 1.0 cm of the object and sniffing or touching the object with its nose, whiskers, or forepaws. Under this definition, a mouse in close proximity to the object without actively attending to it would not be counted as exploration. Discrimination ratios are calculated to account for differences in exploration levels between mice. A ratio value near +1 indicates the animal preferentially explored the novel object (intact recognition memory). A ratio value near –1 means the animal showed more exploration for the familiar object and suggests forgetting had occurred (impaired recognition memory). A score near 0 reflects equal exploration of both objects and no preference for familiar or novel (impaired recognition memory). Statistical analyses were conducted as follows: for all repeated measures (object recognition at 5 min and 24 h, habituation data, place recognition, and metric spatial processing), a two-way ANOVA was performed. For non-repeated measures (configural object recognition), a one-way ANOVA was performed.

Behavioral methods

Object recognition at 5 min—Mice were placed in the center of the red or clear apparatus, presented with two different objects 15 cm apart, and allowed 15 min of free exploration of the apparatus, stimulus objects and distal environmental cues (Study Phase). A black container was placed over the mouse for 5 min (Delay) followed by a Test Phase during which one object was replaced with a new object unfamiliar to the mouse in order to measure object recognition (novel) and the other object was exchanged with an identical copy (familiar). The container was removed and the mouse was allowed to re-explore for 5 min (Test Phase). A pictorial representation of the procedure is shown in Figure 2. The location of the novel object (presented on either the left or right) was counterbalanced in order to avoid spatial preference.

In order to take into account activity level of each mouse for the recall of recognition information, the following Discrimination Ratio was calculated: [Exploration of Novel – Exploration of Familiar]/Total Exploration (Novel + Familiar). Calculated this way, the positive ratio indicated that more time was spent in exploration of the novel stimulus.

Activity level and subsequent habituation for the Ts65Dn/DnJ and control mice in the red and clear box was measured in order to determine whether potential differences during the study phase affected object recognition.

Object recognition at 24 h—The Study Phase is identical to that described for object recognition at 5 min in the red box. The delay period before administering the 5-min Test Phase was 24 h. The discrimination ratio was calculated with the same equation used in the 5-min Object Recognition task.

Place recognition—The Study Phase is the same as in the Object Recognition task, but in the Test Phase one of the objects was located in a different position yet maintaining a distance of 15 cm, in order to measure place recognition (novel) and the other object was exchanged with an identical copy (familiar). A pictorial representation of the procedure is shown in Figure 3. The novel and familiar locations were counterbalanced between trials in order to avoid spatial preference. The Discrimination Ratio was calculated with the same equation used in the Object Recognition task.

Metric spatial processing—The Study Phase is the same as in the Object Recognition task, but the two objects were spaced 25 cm apart. During the Test Phase, identical copies of the same objects were moved closer towards each other so that the objects were now 8 cm apart. A pictorial representation of the procedure is shown in Figure 4. Re-exploration during the Test Phase will be an index of detecting a distance (metric) change. A Discrimination Ratio was calculated: [Test Phase exploration of both objects (A) – last 5 min of Study Phase exploration of both objects (B)]/Total Exploration (A + B). According to this, a positive ratio indicated that more time was spent in exploration of the test phase distance relative to the study phase distance.

Configural object recognition and novelty detection control—This experiment is comprised of two testing conditions, configural object recognition and novelty detection control. 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 (Study Phase). Following a 5 min delay under the black container, the mouse underwent a 5-min Test Phase in which one object from the Study Phase remains 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 is present in one of the compound stimuli in the habituation phase. A pictorial representation of the procedure is shown in Figure 5. Exploration of each compound object was scored as a single unit.

In order to take into account the activity level of each mouse for the recall of recognition information, the following Discrimination Ratio was calculated: [Exploration of Novel (AD) – Exploration of Familiar (AB)]/Total Exploration (AD + AB). In addition to this configural test, a novelty detection condition is necessary to control for the underlying ability to detect novel objects. The Study Phase is identical to the configural test described above, but for the Test phase, the novel stimulus was comprised of two entirely new components (EF), each of

which is novel. Similar to the previous condition, the following Discrimination Ratio was calculated: [Exploration of Novel (EF) – Exploration of Familiar (AB)]/Total Exploration (EF + AB). The order in which animals performed each experimental condition was counterbalanced.

RESULTS

Object Recognition at 5 min

The results for the object recognition tests are shown in Figure 6 and indicate that wild type control mice show good object recognition memory for both the red (minimal contextual information) and clear (contextually rich) boxes. The Ts65Dn/DnJ mice demonstrate intact object recognition, showing a strong preference for the novel object in the red box, but are not able to discriminate between familiar and novel in the clear box. A two-way ANOVA, with groups (Ts65Dn/DnJ and controls) as the between-group factor and boxes (red and clear) as the within-group factor, revealed a significant group effect ($F = 8.86$, $df\ 1,32$, $P = 0.0006$) as well as significant group $\times$ box interaction ($F = 9.36$, $df\ 1,32$, $P = 0.0045$). A subsequent Newman–Keuls paired comparison based on the interaction revealed no significant differences between the performance of Ts65Dn/DnJ and control mice in the red box, but there was a significant difference between the Ts65Dn/DnJ and control mice in the clear box ($P < 0.01$). These results indicate that Ts65Dn/DnJ mice are clearly able to detect novel objects, however they are impaired when there is an important contribution of context.

In addition, activity level and subsequent habituation for the last 10 min (habituation epochs 5–10 and 10–15 min) was measured for the Ts65Dn/DnJ and control mice in the red and clear boxes. The results are shown in Figure 7 and indicate that there is habituation for both groups in the red box and the clear box. A two-way ANOVA was performed with groups (Ts65Dn/DnJ and controls) as the between-group factor and two within-group factors, boxes and time (habituation). There was not a significant group effect nor a significant group $\times$ box interaction, however, there is a significant effect for time ($F = 11.80$, $df\ 1, 32$, $P = 0.002$).

Object Recognition at 24 h

The results of the object recognition test at 24 h are shown in Figure 8 alongside the 5-min test for Ts65Dn/DnJ and control mice and indicate that, when tested at 24 h, the Ts65Dn/DnJ mice are impaired relative to wild type control mice. A two-way ANOVA with groups (Ts65Dn/DnJ and controls) as the between-group factor and time (5 min and 24 h) as the within-group factor revealed a non-significant group effect and a non-significant time effect but a significant group $\times$ time interaction ($F = 9.73$, $df\ 3,47$, $P = 0.0001$). A subsequent Newman–Keuls paired comparison based on the interaction showed that Ts65Dn/DnJ mice, at 24 h, are significantly different from wild type control mice ($P < 0.01$). It should be noted that control mice display object recognition for the 24-h test albeit at a somewhat reduced level compared to the 5-min test.

Place Recognition

The results for the place recognition tests are shown in Figure 9 and indicate that wild type control mice show good place recognition memory in both the red and clear boxes providing different contexts, whereas the Ts65Dn/DnJ mice showed impaired place recognition in both the red and clear boxes. A two-way ANOVA with groups (Ts65Dn/DnJ and controls) as the between-group factor and boxes (red or clear) as the within-group factor revealed a significant group effect ($F = 44.6$, $df\ 1,14$, $P = 0.0001$) but no significant box effect nor a significant box $\times$ group effect. These results imply that Ts65Dn/DnJ mice are clearly impaired when spatial location is important, independent of context.

Metric Spatial Processing

The results of the metric processing tests are shown in Figure 10 and indicate that wild type control mice show good place (metric based) recognition memory for both the red and clear boxes providing different contexts, whereas the Ts65Dn/DnJ mice showed impaired place (metric-based) recognition in both the red and clear boxes. A two-way ANOVA with groups (Ts65Dn/DnJ and controls) as the between-group factor and boxes (red or clear) as the within-group factor revealed a significant group effect ($F = 24.74$, $df\ 1,14$, $P = 0.0002$), but no significant box effect nor a significant box $\times$ group effect. An analysis of wild type performance in the red box relative to a zero baseline revealed that, in the red box, there was a significant difference $t(7) = 2.57$, P (one-tailed) = 0.019, suggesting that the wild type mice detected the novelty of changing the two distances between the two stimuli. These results indicate that Ts65Dn/DnJ mice are clearly impaired when the distance metric for spatial location is important even when independent of context.

Configural Object Recognition and Novelty Control

The results of the configural object recognition test at 5 min are shown in Figure 11 (left) and indicate that both Ts65Dn/DnJ and wild type control mice show good configural object recognition. A one-way ANOVA with the two groups (Ts65Dn/DnJ and controls) revealed no significant group effect ($F = 2.31$, $df\ 1,18$, $P = 0.146$). The results of the control condition for novel object recognition at 5 min are shown in Figure 11 (right) and indicate that Ts65Dn/DnJ and wild type control mice display good novelty detection. A one-way ANOVA with the two groups (Ts65Dn/DnJ and controls) revealed no significant group effect ($F = 2.64$, $df\ 1,18$, $P = 0.122$). These results show that Ts65Dn/DnJ can successfully recognize a novel configuration of familiar elements indicating an ability to detect subtle ambiguities of object configurations (Fig. 11).

DISCUSSION

Based on the observations that the DG in Ts65Dn/DnJ mice reveal a portrait of excessive inhibition within the DG (Kleschevnikov et al., 2012) and that the DG plays an important role in processing spatial location and spatial context information (Goodrich-Hunsaker et al., 2008; Lee et al., 2005; Spanswick and Sutherland, 2010), we hypothesized that Ts65Dn/DnJ mice would be impaired in processing contextual and spatial information as a result of specific dysfunction in the DG and that if object recognition were intact when contextual information was eliminated, this might be a result of preserved function of the PRh. In order

to assess the capability of Ts65Dn/DnJ mice to integrate contextual information and provide behavioral evidence towards DG dysfunction and PRh function, we developed variations of spontaneous novelty detection paradigms with or without the presence of contextual cues, thereby isolating novelty awareness from integration of spatial information. The summary of results (Figure 12) support the hypothesis that deficits of DG and relative preservation of PRh when contextual interference is minimized in the Ts65Dn mouse model of DS.

The results of the first experiment with Ts65Dn/DnJ in the object recognition task revealed that Ts65Dn/DnJ mice were not impaired at short delays (5 min) in the red box (minimal contextual information), but were impaired in the clear box where visual cues surrounding the apparatus provided a spatial context. Furthermore, there are no overall differences in habituation as a function of decreased object exploration over time, although it does appear that there was very little habituation for the Ts65Dn/DnJ mice in the clear box, suggesting that the somewhat increased exploration could be due to attempts to process the external context. Therefore, changes in activity level and habituation during the test phase cannot account for differential performance of Ts65Dn/DnJ mice in the red and clear boxes. The data are consistent with findings of Braudeau et al. (2011) who also found a deficit for Ts65Dn/DnJ at short delays in an open field box. The current data suggest the Ts65Dn/DnJ can perform as well as controls on short-term recognition tests. The results are consistent with the finding that DG lesions made with colchicine or induced by adrenalectomy result in normal performance on object recognition (Lee et al., 2005). An intriguing possibility is that the PRh is in part, intact in the Ts65Dn/DnJ mice because PRh lesions do impair object recognition (Brown and Aggleton, 2001; Winters et al., 2010; Spanswick and Sutherland, 2010). In the clear box where a spatial context is comprised of visual cues around the box, a deficit emerges for the Ts65Dn/DnJ mice. These data are consistent with the findings of Spanswick and Sutherland (2010). The importance of large HC lesions, which often include DG lesions, in mediating the contribution of a spatial context in object recognition tasks has been shown in a number of studies (Mumby, 2001; Langston and Wood, 2010). Taken together, the data indicate dysfunction of the DG and preserved function of the PRh in the Ts65Dn mouse.

The results of the second and third experiment with Ts65Dn/DnJ in the place recognition and metric spatial task revealed that Ts65Dn/DnJ mice were impaired at short delays (5 min) in both the red and clear box, suggesting that the representation of spatial location information is also impaired in Ts65Dn/DnJ mice. These data are consistent with the observation that lesions of the DG (but not CA1, CA3, or the PRh) disrupt place recognition (Lee et al., 2005) and metric spatial processing (Goodrich-Hunsaker et al., 2008). It should be noted that in some studies DG lesions have not produced a deficit in spatial recognition memory (Hernández-Rabaza et al., 2007; Spanswick and Sutherland, 2010). In summary, these tests of place and metric processing support the hypothesis that DG dysfunction is a major contributor to impaired spatial as well as contextual processing shown above in the Ts65Dn/DnJ mouse model of DS.

The results of the fourth experiments with Ts65Dn/DnJ in the object recognition task revealed that Ts65Dn/DnJ mice were impaired at long delays (24 h) in the red box. The data are consistent with findings of Faizi et al. (2011), Fernandez et al. (2007), and Lockrow et

al. (2010), who also found a deficit for Ts65Dn/DnJ mice at long delays (24 h) in a closed box. Despite the fact that the above mentioned data are not consistent with their observation of a lack of deficit at the 5-min test, the possibility exists that the feed forward effect of a DG dysfunction on CA1 could result in problems in processing object information that needs to be consolidated across a 24-h period. For a review, see Rolls and Kesner (2006).

The results of the last experiment, the configural object recognition task, revealed that Ts65Dn/DnJ performance did not significantly differ from controls in their ability to recognize the novel stimulus at short delays (5 min) in the red box, which had a novel configuration of familiar elements and thus recognition could only be facilitated through the use of configural representations. In addition, Ts65Dn/DnJ performance did not significantly differ from wild type mice on the control condition for novelty detection at short delays (5 min) in the red box, when stimuli can be recognized as novel based solely on individual elements. The data are consistent with the results from short/long term object recognition tests discussed above showing Ts65Dn/DnJ have no impairment at short-term object recognition in the absence of contextual cues. The ability of Ts65Dn/DnJ to show preference for a novel configural arrangement of familiar stimuli can be viewed as an ability to resolve feature ambiguity, a function that depends on the PRh to form conjunctive configural representations in situations of high feature ambiguity. This subsequence study provides further support for the hypothesis that in the Ts65Dn/DnJ, PRh function may be, at least in part, preserved.

These experiments illustrate that HC function is impaired under two conditions; when consolidation is required for long-term 24-h recognition memory and when contextual cues are available. They do not show that object recognition is impaired, a function possibly spared by a working PRh. It is notable that the pattern of results so far are consistent with deficits observed in rats with DG lesions (Deese and Kesner, 2013), supporting the hypothesis of a dysfunction of the DG in Ts65Dn mice (Fernandez et al., 2007). Together, these data suggest that the DG represents one of the regions with specific dysfunction, and the PRh with specific preserved function, in DS mice and perhaps also in DS individuals.

One way to explain the results is based on the idea that, in the object recognition task in the clear box, there is a potential for spatial interference between local object cues and the cue-context cues that surround the clear box. It is known that one of the functions of the DG is to decrease spatial interference which has been described as a spatial location and spatial context pattern separation process (Gilbert et al., 2001; Hunsaker and Kesner, 2013; Kesner, 2013). Thus, control rats with an intact DG can reduce the interference between local object cues and the cue-context cues that surround the clear box and display high levels of exploratory behavior for detecting the change of one of the objects resulting in good object recognition. In contrast, rats with DG lesions experience too much interference between local object cues and the cue-context cues that surround the clear box, resulting in difficulty detecting the object change and poor object recognition.

Current evidence suggests that the HC supports high-level contextual integration of spatial representations. On the other hand, the PRh supports high-level conjunctive representations of simple features from visual stimulus (Kealy et al., 2011). Another way to look at it is that

the HC performs spatial pattern separation/completion (situations of ambiguity occurring in spatial discriminations) while the PRh performs visual discriminations (situations of feature ambiguity). Double dissociation studies have confirmed that animals with PRh lesions are impaired on object recognition but not spatial memory tasks, and animals with hippocampal lesions are impaired on spatial but not object recognition memory tasks (Kealy et al., 2011). Furthermore, Aggleton and Brown (2006) maintain that there is evidence to support that the familiarity discrimination mechanisms in the PRh are related to individual stimuli, and do not deal with the novel arrangement of familiar spatial stimuli, nor with associative and contextual aspects of recognition memory. It is also known that rats automatically learn the spatial disposition of objects explored in an open-field arena (Poucet, 1989; Dix and Aggleton, 1999). Taken together, this implies that the PRh could support object recognition through conjunctive visual representations, but if contextual information is available (such as in the presence of distal environmental cues) or spatial representations are required, the automatic integration of object with context would require the involvement of HC networks. For a comprehensive review of functional, anatomical and physiological evidence for the segregation within the PRh and as part of a hippocampal–parahippocampal network, see Kealy and Commins (2011).

In summary, it appears that the Ts65Dn/DnJ mice have a dysfunction of the DG with concomitant problems in processing spatial and contextual information associated with DG function. Future studies utilizing additional variations of mouse models of DS can be crucial to understanding the contribution of individual genes to learning and memory phenotypes of DS. Even though previous studies have emphasized the importance of the hippocampus in supporting cognitive functions in DS patients, the present study strongly implicates specific dysfunction of the DG as a contributing factor to DS cognitive deficits and therefore the DG is a specific therapeutic target for future approaches. A parallel set of tasks in humans may also increase the efficacy of cognitive therapeutics developed in mouse for human cognition.

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REFERENCES

- Aggleton JP, Brown MW. Interleaving brain systems for episodic and recognition memory. *Trends Cogn Sci.* 2006; 10:455–463. [PubMed: 16935547]
- Bartko SJ, Winters BD, Cowell RA, Saksida LM, Bussey TJ. Perirhinal cortex resolves feature ambiguity in configural object recognition and perceptual oddity tasks. *Learn Mem.* 2007; 14:821–832. [PubMed: 18086825]
- Belichenko PV, Kleschevnikov AM, Masliah E, Wu C, Takimoto-Kimura R, Salehi A, Mobley WC. Excitatory-inhibitory relationship in the fascia dentate in the Ts65Dn mouse model of Down syndrome. *J Comp Neurol.* 2009; 512:453–466. [PubMed: 19034952]
- Belichenko PV, Masliah E, Kleschevnikov AM, Villar AJ, Epstein CJ, Salehi A, Mobley WC. Synaptic structural abnormalities in the Ts65Dn mouse model of Down syndrome. *J Comp Neurol.* 2004; 480:281–298. [PubMed: 15515178]

- Braudeau J, Delatour B, Duchon A, Pereira PL, Dauphinot L, de Chaumont F, Olivo-Marin JC, Dodd RH, Héroult Y, Potier MC. Specific targeting of the GABA-A receptor $\alpha 5$ subtype by a selective inverse agonist restores cognitive deficits in Down syndrome mice. *J Psychopharmacol.* 2011; 25:1030–1042. [PubMed: 21693554]
- Brown MW, Aggleton JP. Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci.* 2001; 2:51–61. [PubMed: 11253359]
- Deese BS, Kesner RP. The role of the dorsal dentate gyrus in object and object-context recognition. *Neurobiol Learn Mem.* 2013 (in press).
- Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. *Behav Brain Res.* 1999; 99:191–200. [PubMed: 10512585]
- Ennaceur A. One trial object recognition in rats and mice: Methodological and theoretical issues. *Behav Brain Res.* 2010; 215:244–254. [PubMed: 20060020]
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res.* 1988; 31:47–59. [PubMed: 3228475]
- Faizi M, Bader PL, Tun C, Encarnacion A, Kleschevnikov A, Belichenko P, Saw N, Priestley M, Tsien RW, Mobley WC, Shamloo M. Comprehensive behavioral phenotyping of Ts65Dn mouse model of Down syndrome: Activation of $\beta 1$ -adrenergic receptor by xamoterol as a potential cognitive enhancer. *Neurobiol Dis.* 2011; 43:397–413. [PubMed: 21527343]
- Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC. Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat Neurosci.* 2007; 10:411–413. [PubMed: 17322876]
- Gilbert PE, Kesner RP, Lee I. Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1. *Hippocampus.* 2001; 11:626–636. [PubMed: 11811656]
- Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP. The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behav Neurosci.* 2008; 122:16–26. [PubMed: 18298245]
- Hernández-Rabaza V, Barcia JA, Llorens-Martín M, Trejo JL, Canales JJ. Spared place and object-place learning but limited spatial working memory capacity in rats with selective lesions of the dentate gyrus. *Brain Res Bull.* 2007; 72:315–323. [PubMed: 17452292]
- Hunsaker M, Kesner RP. The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neurosci and Biobehav Rev.* 2013; 37:36–58. [PubMed: 23043857]
- Kealy J, Commins S. The rat perirhinal cortex: A review of anatomy, physiology, plasticity, and function. *Prog Neurobiol.* Apr; 2011 93(4):522–48. 2011. [PubMed: 21420466]
- Kesner, RP.; Morris, AM.; Weeden, CS. Spatial, temporal, and associative behavioral functions associated with different subregions of the hippocampus. In: Zentall, TR.; Wasserman, EA., editors. *The Oxford Handbook of Comparative Cognition.* Oxford University Press; Oxford, UK: 2012. p. 322-344.
- Kesner RP. Role of the hippocampus in mediating interference as measured by pattern separation. *Behavioral Processes.* 2013; 93:148–154.
- Kleschevnikov AM, Belichenko PV, Gall J, George L, Nosheny R, Maloney MT, Salehi A, Mobley WC. Increased efficiency of the GABAA and GABAB receptor-mediated neurotransmission in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis.* 2012; 45:683–691. [PubMed: 22062771]
- Langston RF, Wood ER. Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus.* 2010; 20:1139–1153. [PubMed: 19847786]
- Lee I, Hunsaker MR, Kesner RP. The role of hippocampal sub-regions in detecting spatial novelty. *Behav Neurosci.* 2005; 119:145–153. [PubMed: 15727520]
- Lockrow D, Boger H, Bimonte-Nelson H, Granholm A. Effects of memantine on memory and neuropathology in Ts65Dn mice. *Behav Brain Res.* 2010; 221:610–622. [PubMed: 20363261]
- Mumby DG. Perspectives on object-recognition memory following hippocampal damage: Lessons from studies in rats. *Behav Brain Res.* 2001; 127:159–181. [PubMed: 11718890]

- Popov VI, Kleschevnikov AM, Klimenko OA, Stewart MG, Belichenko PV. Three-dimensional synaptic ultrastructure in the dentate gyrus and hippocampal area CA3 in the Ts65Dn mouse model of Down syndrome. *J Comp Neurol*. 2011; 519:1338–1354. [PubMed: 21452200]
- Poucet B. Object exploration, habituation, and response to a spatial change in rats following septal or medial frontal cortical damage. *Behav Neurosci*. 1989; 103:1009–1016. [PubMed: 2803548]
- Rolls ET, Kesner RP. A computational theory of hippocampal function, and empirical tests of the theory. *Prog Neurobiol*. 2006; 79:1–48. [PubMed: 16781044]
- Seregaza Z, Roubertoux PL, Jamon M, Soumireu-Mourat B. Mouse models of cognitive disorders in trisomy 21: A review. *Behav Genet*. 2006; 36:387–404. [PubMed: 16523244]
- Spanswick SC, Sutherland RJ. Object/context-specific memory deficits associated with loss of hippocampal granule cells after adrenalectomy in rats. *Learn Mem*. 2010; 17:241–245. [PubMed: 20410060]
- Winters BD, Saksida LM, Bussey TJ. Implications of animal object memory research for human amnesia. *Neuropsychologia*. 2010; 48:2251–2261. [PubMed: 20132832]

FIGURE 1.
Behavioral apparatus with and without contextual cues.

FIGURE 2.
Schematic of object recognition paradigm.

FIGURE 3.
Schematic of place recognition paradigm.

FIGURE 4.
Schematic of metric spatial processing paradigm.

FIGURE 5.
Schematic of configural object recognition paradigm.

FIGURE 6.

Object recognition at 5 min for red (minimal context) and clear (contextually rich) boxes in Ts65Dn/DnJ and control mice. Discrimination between novel and familiar objects based on exploration time during test phase is presented as mean discrimination ratio $\pm$ STE. For all groups, $n = 17$ (* $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$).

FIGURE 7.

Exploration for the last 10 min of the habituation phase was measured for Ts65Dn/DnJ mice and control mice in the red (minimal context) and clear (contextually rich) boxes. For all groups, $n = 17$.

FIGURE 8.

Object recognition in the red box (minimal context) at 5 min and 24 h in Ts65Dn/DnJ and control mice. Discrimination between novel and familiar objects based on exploration time during test phase is presented as mean discrimination ratio $\pm$ STE. Ts65Dn/DnJ and control mice at 5 min, $n = 17$; Ts65Dn/DnJ at 24 h, $n = 8$; Wild type controls at 24 h, $n = 9$ (* $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$).

FIGURE 9.

Place recognition for red (minimal context) and clear (contextually rich) boxes in Ts65Dn/DnJ and control mice. Discrimination between novel and familiar locations based on exploration time during test phase is presented as mean discrimination ratio $\pm$ STE in the red and clear box. For all groups, $n = 8$ (* $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$).

FIGURE 10.

Metric Spatial Processing for red (minimal context) and clear (contextually rich) boxes in Ts65Dn/DnJ and control mice. Discrimination between novel and familiar metric distances based on exploration time during test phase and last 5 min of study phase is presented as mean discrimination ratio $\pm$ STE. For all groups, $n = 8$. (* $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$).

FIGURE 11.

Configural object recognition and Novelty detection tests in the red box (minimal context) in Ts65Dn/DnJ and control mice. Discrimination between novel and familiar objects based on exploration time during test phase is presented as mean discrimination ratio $\pm$ STE. For all conditions, $n = 10$.

	Clear Box		Red Box	
	Ts65Dn	Control	Ts65Dn	Control
Object Recognition at 5min	-	+	+	+
Object Recognition at 24 hours	n/a	+	-	+
Place Recognition	-	+	-	+
Metric Recognition	-	+	-	+
Configural Object Recognition	n/a	+	+	+

+ = intact performance - = impaired performance

FIGURE 12.

Table summarizing performance of Ts65Dn/DnJ and wild type control mice on all tasks under conditions of minimal context (Red Box) or rich context (Clear Box). Minimizing contextual information interference facilitates object recognition and ambiguity discrimination in the Ts65Dn mouse.