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Research paper

Suprachiasmatic lesions restore object recognition in down syndrome model mice

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ABSTRACT

The Ts65Dn mouse is a well-studied model of trisomy 21, Down syndrome. This mouse strain has severe learning disability as measured by several rodent learning tests that depend on hippocampal spatial memory function. Hippocampal long-term potentiation (LTP) is deficient in these mice. Short-term daily treatment with low-dose GABA receptor antagonists rescue spatial learning and LTP in Ts65Dn mice leading to the hypothesis that the learning disability is due to GABAergic over-inhibition of hippocampal circuits. The fact that the GABA receptor antagonists were only effective if delivered during the daily light phase suggested that the source of the excess GABA was controlled directly or indirectly by the circadian system. The central circadian pacemaker of mammals is the suprachiasmatic nucleus (SCN), which is largely a GABAergic nucleus. In this study we investigated whether elimination of the SCN in Ts65Dn mice would restore their ability to form recognition memories as tested by the novel object recognition (NOR) task. Full, but not partial lesions of the SCN of Ts65Dn mice normalized their ability to perform on the NOR test. These results suggest that the circadian system modulates neuroplasticity over the time frame involved in the process of consolidation of recognition memories.

1. Introduction

Several mouse models of Down syndrome (DS) or Trisomy 21 exist. Here we have decided to study the Ts65Dn mouse because it is a well-established model that has been studied extensively as an animal model of DS (Reeves et al., 1995; Holtzman et al., 1996; Olson et al., 2004; Rueda et al., 2012; Fernandez et al., 2007; Fernandez and Garner, 2007; Colas et al., 2013). It carries a reciprocal translocation that is trisomic for approximately 104 genes (56%) on Mmu16 that have homologues on HSA21 (Reeves et al., 1995). Moreover, this model recapitulates many of the neuroanatomical and behavioral alterations of DS seen in humans including learning and memory deficits. Electrophysiological studies had implicated deficiencies in hippocampal LTP in these mice and pointed to excessive GABAergic inhibition as a possible causative factor (Siarey et al., 1997, 1999; Kleschevnikov et al., 2004; Costa and Grybko, 2005). That possibility was born out by studies showing that chronic, but short term, low dose treatments with GABA receptor antagonists normalized LTP in Ts65Dn mice and also normalized their performance on rodent memory tasks – novel object recognition (NOR) and spontaneous alternation in a T-maze (SA) (Fernandez et al., 2007). A remarkable finding in that and following studies (Colas et al. 2013, 2017) is that a short-term (2 week), daily treatment with low dose GABA receptor antagonists results in long-term, greater than 2 months, normalization of the learning ability of the mice. The obvious question following the studies with GABA receptor antagonists was what is the source of the excessive GABAergic activity.

$GABA_A$ receptors are much involved in sleep (Gottesman, 2002) and in circadian rhythms (Saper et al., 2005; DeWoskin et al., 2015). Sleep and circadian rhythms are both involved in learning and memory (Rasch and Born, 2013; Smarr et al., 2014). Therefore, we asked if abnormalities in either circadian rhythms or sleep could underlie the learning disability of Ts65Dn mice. Comparisons of sleep architecture in two mouse models of DS, Ts65Dn and Ts1Cje, with each other and with 2N controls were carried out by Colas et al. (2008). Ts1Cje mice showed no differences from their 2N littermates, but Ts65Dn mice showed more wake and less NREM sleep during their active phases. The Ts65Dn mice also showed higher EEG power in the theta band during sleep, and this difference was not seen in the Ts1Cje mice. Although small, these

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differences in sleep characteristics may be significant in light of the fact that the Ts1Cje mice do not show deficits in novel object memory (Fernandez and Garner, 2007). However, the primary differences in sleep were found during the active phase in the Ts65Dn mice, and not in the sleep phase when memory consolidation processes are ongoing.

We also asked whether treatment with the GABA antagonist pentylenetetrazole (PTZ) had any effect on the circadian rhythms of the mice (Ruby et al., 2010). Our metric was activity. The circadian rhythms of the Ts65Dn mice were robust with only minor differences from their 2N littermates. The Ts65Dn mice were more active at night, and when moved from a light/dark (LD) cycle to constant dark, they remained more active during the subjective night. On the LD cycle the Ts65Dn mice had longer alphas (activity phases), greater rhythm power, and greater total activity. In constant dark, there was a significantly higher rhythm power in the Ts65Dn mice. There were no effects of PTZ on the rhythm characteristics of either the Ts65Dn mice or their 2N littermates. Definitely, the Ts65Dn mice did not have obvious deficiencies in their circadian rhythms.

Potential significance of strong circadian rhythms in the Ts65Dn mice was suggested by results of an extensive preclinical study of PTZ as a potential pro-cognitive therapeutic for DS (Colas et al., 2013). That study showed a strong time of day effect on the efficacy of PTZ dosing. If PTZ dosing occurred during the dark phase, unlike the light phase, it had no beneficial effect on the spatial memory performance of the Ts65Dn mice. Since the SCN is a GABAergic nucleus (DeWoskin et al., 2015), and since the SCN activity is always higher in the light phase than in the dark phase (Schwartz et al., 1983), we hypothesized that PTZ was possibly acting directly or indirectly on the circadian system to reduce over-inhibition on hippocampal circuits.

The possibility of the SCN as a source of inhibition of neuroplasticity was supported by our previous work on learning and memory in the Siberian hamster. In that animal model, a simple light treatment would render the animals circadian arrhythmic (Ruby et al., 1996), and once arrhythmic, they were learning impaired (Ruby et al., 2008). As in the Ts65Dn mouse, short-term chronic treatment with PTZ restored learning in these animals, but not their rhythms (Ruby et al., 2013). The remarkable finding was that if the SCNs of these arrhythmic hamsters were lesioned, their ability to perform on learning and memory tasks was restored (Fernandez et al., 2014). Taking the results from the hamster studies and the Ts65Dn mouse studies together, either continuous activity or over-activity of the SCN seemed to impair learning.

The studies cited above showing hippocampal LTP impairment in Ts65Dn mice and its normalization by PTZ treatment suggest that the potential site of SCN action is hippocampal circuits. However, the SCN does not project to the hippocampus, so its action in modulating neuroplasticity would have to be indirect as suggested previously by our laboratory (Ruby et al., 2008). The SCN does project to the septum, which in turn exerts influence over the hippocampus (Watts et al., 1987). Injections of $GABA_A$ receptor agonists into the septum disrupt performance in hippocampal dependent learning tasks (Parent et al., 1997; Degroot and Parent, 2001; Krebs and Parent, 2005). If our reasoning that the SCN is exerting an indirect inhibition on neuroplasticity is correct, then lesions of the SCN should eliminate the GABAergic over-inhibition of hippocampal circuits, and learning ability of Ts65Dn mice should be improved.

2. Materials and methods

2.1. Animals and genotyping

Segmental trisomic 16 (Ts65Dn) mice were obtained by mating female carriers of the 17^{16} chromosome (B6EiC3H – a/ATs65Dn) with C57BL/6J Ei × C3H/HeSnJ (C3H) F1 hybrid males (Reeves et al., 1995) and produced either at Jackson West Laboratories, (Davis, CA) or in our colony. Mice used in our studies were Ts65Dn mice (TS) on the B6/C3H background with diploid (2N) littermates as controls. Mice were maintained at 23 ± 2 °C on a 12:12 Light-Dark schedule and had access to food and water *ad libitum*. All experimental procedures were approved by the Stanford University IACUC and were conducted in compliance with the NIH Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize the number of animals used and to minimize their discomfort as our aim was to study the behavior of non-stressed animals. The 2N and Ts65Dn mice were genotyped using real-time quantitative PCR with *App*- and *Apob*-specific TaqMan probes (Applied Biosystems). Mice carrying the retinal degeneration (*Rd*) allele were excluded from experiments (Blank et al., 2011). Mice were randomly assigned to experimental groups.

2.2. Activity recording and analysis

For assessment of circadian rhythmicity, mice were maintained in constant dark and their activity bouts were summed in 10-min intervals by passive infrared motion detectors. Activity data were evaluated by chi-square periodogram analysis (ClockLab, Actimetrics, Evanston, IL) on 10-day blocks of data for each animal prior to baseline behavioral testing, and following SCN lesion surgery. Peaks in the periodogram were deemed statistically significant if they exceeded the 99.9% confidence interval limit. Animals were considered arrhythmic if there were no significant peaks in the periodogram in the circadian range, activity was distributed throughout the Light/Dark cycle, and if daily rhythm onsets and offsets could not be identified visually. The time of day when animals were tested is given by zeitgeber time (ZT) where ZT 6 = time of lights-on and ZT 18 = time of lights-off in the animal room.

2.3. Behavioral testing

The behavioral test used in this study for assessing long-term memory was novel object recognition or NOR (Dere et al., 2007) carried out in arenas (50 × 50 × 50cm) resting on an infra-red emitting base. Behavior was recorded by an infrared-sensitive camera placed 2.5m above the arenas. Behavioral testing was carried out in dim light within 2h in the middle of the light phase (6h after light onset). Data were stored and analyzed using Videotrack software from ViewPoint Life Sciences, Inc. (Montreal, Canada). On the day before NOR training, mice were habituated to the open arenas. NOR is based on propensity of mice to explore a novel object versus a previously experienced object when allowed to explore freely. Procedures described in Colas et al. (2013) were used: for NOR training, two identical objects were placed in the arena and animals were allowed to explore them for 10 min. Testing occurred 24 h later in the same arena, but one of the original objects used during training was replaced by a novel object. Objects were of similar dimensions, and prior testing showed that they did not elicit spontaneous preferences (Supplementary Fig. 1). However, to avoid any inherent preference for one object we also randomly presented the objects as familiar or the new one. Testing sessions were 7min after which the objects and arenas were cleaned with 10% ethanol. Object exploration was measured by time spent with the nose directed at and within 2.5 cm from the object. A learning index (LI) for each animal in each trial was calculated as ratio of time spent exploring the novel object over total time spent exploring both objects x 100%. The LI is calculated as the cumulative time spent with objects over 10 min (training) or over 7 min (testing). The LIs were averaged among the groups of mice by genotype/treatment/condition. The LIs should be non-significantly different from 50% in the training session, and significantly increased in test sessions *vs.* training sessions if novelty is detected (Colas et al., 2013). We also measured the exploration time (time spent close to the two objects) and the distance travelled in the arena during the NOR.

2.4. SCN lesions

Males and females were used in all groups and were 5–6 months of

age at the start of the experiment. Mice were separated and housed singly. For SCN lesion surgery, mice were anesthetized with isoflurane. The skull was secured and held level in a stereotaxic apparatus. The top of the head was shaved and swabbed three times with betadine followed by alcohol. A longitudinal incision was made on midline, the skin retracted, and the surface of the skull scraped and swabbed with H_2O_2. Small holes were drilled through the skull 0.35 mm anterior to bregma, ± 0.15 mm lateral to midline. A flexible stainless-steel electrode (Neurotherm, Wilmington, MA) that was insulated except for 2 mm at its tip was lowered bilaterally 5.7 mm ventral to dura. Lesions were created by heating the electrode tip to 55 ˚C for 10 s. Sham-operated animals underwent the same procedure except the lesions spared all or part of the SCN.

2.5. Lesion verification

Brains were removed, and frozen coronal sections (30 μm) were cut through the area of the optic chiasm. Mounted sections were stained with cresyl violet, and the extent of the damage was assessed microscopically. Histological evaluation of tissue damage was performed by an independent investigator (N.F.R.) without knowledge of the corresponding behavioral data.

2.6. Statistical analysis

Data are presented as mean ± S.E.M. and were analyzed using GraphPad Prism (San Jose, CA). NOR data were analyzed by comparing mean LIs in testing *vs.* training sessions using a two-tailed t-test for paired samples for each genotype/treatment group. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Brain lesions

SCN lesions were defined as having no visible SCN tissue remaining. Ts65Dn mice that sustained complete ablation of the SCN (CL) also sustained ablation of the hypothalamic paraventricular nucleus, preoptic area, anterior and lateral hypothalamus, subparaventricular zone, and retrochiasmatic area. There was also varying degrees of damage to the septal nuclei, bed nucleus of the stria terminalis, and ventromedial thalamus, but damage to these areas was not consistent among animals.

In mice that sustained only partial ablation of the SCN (PL), lesions appeared to be centered anterior and dorsal to the SCN, thus damage was confined to the dorsal/rostal regions of the nucleus. Damage outside the SCN was mainly limited to the preoptic area and anterior hypothalamus, as well as the ventral paraventricular nucleus. The optic chiasm and tracts were observed to be intact during brain removal and tissue sectioning (See Fig. 1).

3.2. 2N and Ts65Dn comparison at baseline

3.2.1. Activity profile at baseline

Prior to lesion surgeries all of the Ts65Dn and 2N mice had robust light/dark patterns in locomotor activity (Fig. 2A, *$p < 0.05$), as well as free-running circadian rhythms of activity (Fig. 3A, left). The 24 h. activity records showed that the Ts65Dn mice tended to be more active than the 2N mice (Fig. 2A; $t = -1.881$, $p < 0.07$).

3.2.2. Novel object recognition (NOR) at baseline

The inability of Ts65Dn mice to perform in the novel object recognition (NOR) test in our laboratory was documented in our earlier work (Colas et al., 2013). Nevertheless, we confirmed that result with the current lineage of Ts65Dn mice being used in this study. Seventeen 2N mice and eleven of their Trisomic (Ts65Dn) littermates were trained in the NOR protocol during the second half of the light phase and tested 24 h. later. Whereas the 2N mice showed recognition of the novel object, their Ts65Dn littermates did not (Fig. 2B; 2N: $t = -5.319$, $p < 0.0001$; Ts: $t = -1.183$, $p = 0.2643$).

Because amount of contact with the objects might influence the ability of the mice to remember the objects, we quantified the activity of the animals and the time spent close to the new object. Unlike the Ts65Dn mice, the 2N mice spent more time exploring the objects during testing than during training (2N: $t = -2.821$, $p = 0.01$; Ts: $t = -0.684$, $p = 0.51$). The Ts65Dn mice tented to explore the objects more than the 2N mice during training ($t = -1.971$, $p < 0.06$) but not during the testing ($t = -1.247$, $p = 0.22$, Fig. 2B).

Consistent with the 24 h. activity recordings, the Ts65Dn mice were significantly more active than the 2N controls in the arenas used for NOR testing ($t = -2.296$, $p = 0.03$) but not during the NOR training ($t = -1.723$, $p < 0.10$). For both groups of mice there were no differences in distance travelled between NOR training and testing intervals (Fig. 2B, 2N: $t = 0.712$, $p = 0.49$; Ts: $t = -1.214$, $p = 0.25$).

3.3. Profile of activity after SCN lesion

Following surgeries, 12 Ts65Dn and 19 2N littermate mice were rendered arrhythmic by the lesions (Fig. 3A). The surgery significantly decreased activity in Ts65Dn mice ($t = 2.300$, $p = 0.03$), but did not change the activity of the 2N mice ($t = 1.059$, $p = 0.31$). No differences regarding activity were observed between 2N and Ts mice (Rhy: $t = -1.141$, $p = 0.26$; Arr: $t = 0.358$, $p = 0.72$, Fig. 3B).

3.4. NOR after lesion

Following SCN lesion surgery, all 2N mice (intact ($n = 6$, $t = 3.16$, $p = 0.025$), partial lesions ($n = 6$, $t = 2.99$, $p = 0.031$), and complete lesions ($n = 9$, $t = 8.41$, $p < 0.001$) showed a preference for the new object in the 24 h. NOR test with scores that were significantly different from 50% (Fig. 4A). The Ts65Dn mice that remained rhythmic continued to show impaired learning in the 24 h. NOR testing (a learning index around 50%, Fig. 4A). The Ts65Dn mice that were rendered arrhythmic, and subsequently shown to have complete SCN lesions ($n = 5$), showed significant improvement in the NOR test ($t = 3.62$, $p = 0.022$), whereas Ts65Dn mice with partial lesions ($n = 5$, $t = 0.64$, $p = 0.556$) or that were left neurologically intact ($n = 12$, $t = 1.17$, $p = 0.265$) failed at the NOR test (Fig. 3Figure 4A). There were no differences between the three Ts65Dn groups on day 1 – training day ($p > 0.05$).

We also recorded total time spent exploring the new object and the activity of the mice during NOR training and testing following the SCN surgeries (Fig. 4B. and C.). A 2-way ANOVA revealed a significant effect of lesion group ($F_{(2, 19)} = 12.42$, $p = 0.0004$, Fig. 4B). The 2N with partial or complete lesion of the SCN showed a large increase in exploration time (2-way ANOVA: effects for "group": $F_{(2,19)} = 12.42$, $p < 0.001$; Sidak's post-hoc comparisons: Intact vs. CL, $p < 0.001$, Intact vs. PL, $p = 0.005$), but no group had a difference in exploration time between training (D1) and testing (D2) (effects for "day": $F_{(1,19)} = 0.195$, $p = 0.664$). By contrast, no such differences were found among the Ts65Dn groups (2-way ANOVA, all comparisons, $p > 0.05$). Among the 2N mice, the CL group travelled significantly further than the 2N intact group on day 1 (2-way ANOVA: effect for "group": $F_{(5,37)} = 2.46$, $p = 0.051$; Sidak's post-hoc comparisons: day 1 2N Intact vs. CL, $p = 0.022$, Fig. 4C), but not on day 2 ($p > 0.05$). There were no significant differences between groups for the Ts65Dn mice (right) on days 1 or 2 ($p > 0.05$).

4. Discussion

The Ts65Dn mice in our study performed poorly on the NOR test as reported previously (Colas et al. 2013, 2017), whereas their 2N littermates showed a clear ability to recognize novel objects 24 h. after short

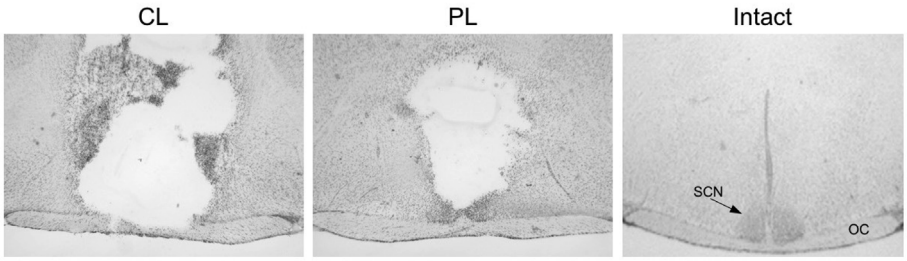

Fig. 1. Tissue sections from mice with complete (CL) or partial lesions (PL) of the SCN, and from an unlesioned intact animal (Intact). The SCN is indicated by the arrow. Optic chiasm (OC) is shown for reference.

exposure to training objects. This apparent cognitive disability of the Ts65Dn mice could not be attributed to lack of exploratory activity or even time spent in contact with the objects. In baseline testing the Ts65Dn mice were more active than the 2N mice and spent more time exploring the objects. It is also unlikely that the poor NOR performance of the Ts65Dn mice was due to anxiety. Even when undisturbed in their home cages, the Ts65Dn mice had higher levels of activity than did their 2N littermates, but when their behaviors were compared in open field testing in the NOR test arenas, both 2N and Ts65Dn mice spent the same percentage of time in the center area of the arena (Supplementary Fig. 2). The NOR disability of the Ts65Dn mice is a robust characteristic of these animals, and it appears to be reflected in measures of hippocampal LTP (Siarey et al., 1997, 1999; Kleschevnikov et al., 2004; Costa and Grybko, 2005).

The LTP studies suggested that the mechanistic cause of the cognitive deficits in the Ts65Dn mice was excessive GABAergic inhibition. That possibility was convincingly borne out by experiments showing that treatments of the Ts65Dn mice with GABA receptor antagonists restored their abilities to perform on NOR testing as well as their 2N littermates (Fernandez et al., 2007; Colas et al. 2013, 2017). The remarkable finding from these studies was that a short-term (2 week) course of daily, low dose administrations of the GABA receptor antagonists resulted in long-term restoration of their NOR performance abilities. However, the treatment was only successful if the drugs were administered during the light-phase of the animals' daily rest/activity cycles. These results suggested involvement of the circadian system in the NOR disability of the Ts65Dn mice.

Circadian rhythms have been shown in many studies to play important roles in learning and memory (see Smarr et al., 2014 for a review). These studies extend to effects of chronic jet lag on humans (Cho et al., 2000; Cho, 2001) that produce cognitive deficits. Whereas many protocols resulting in disruption of circadian rhythms are shown to impair cognitive performance, experiments in which circadian rhythms are eliminated by SCN lesion do not seem to have negative effects on

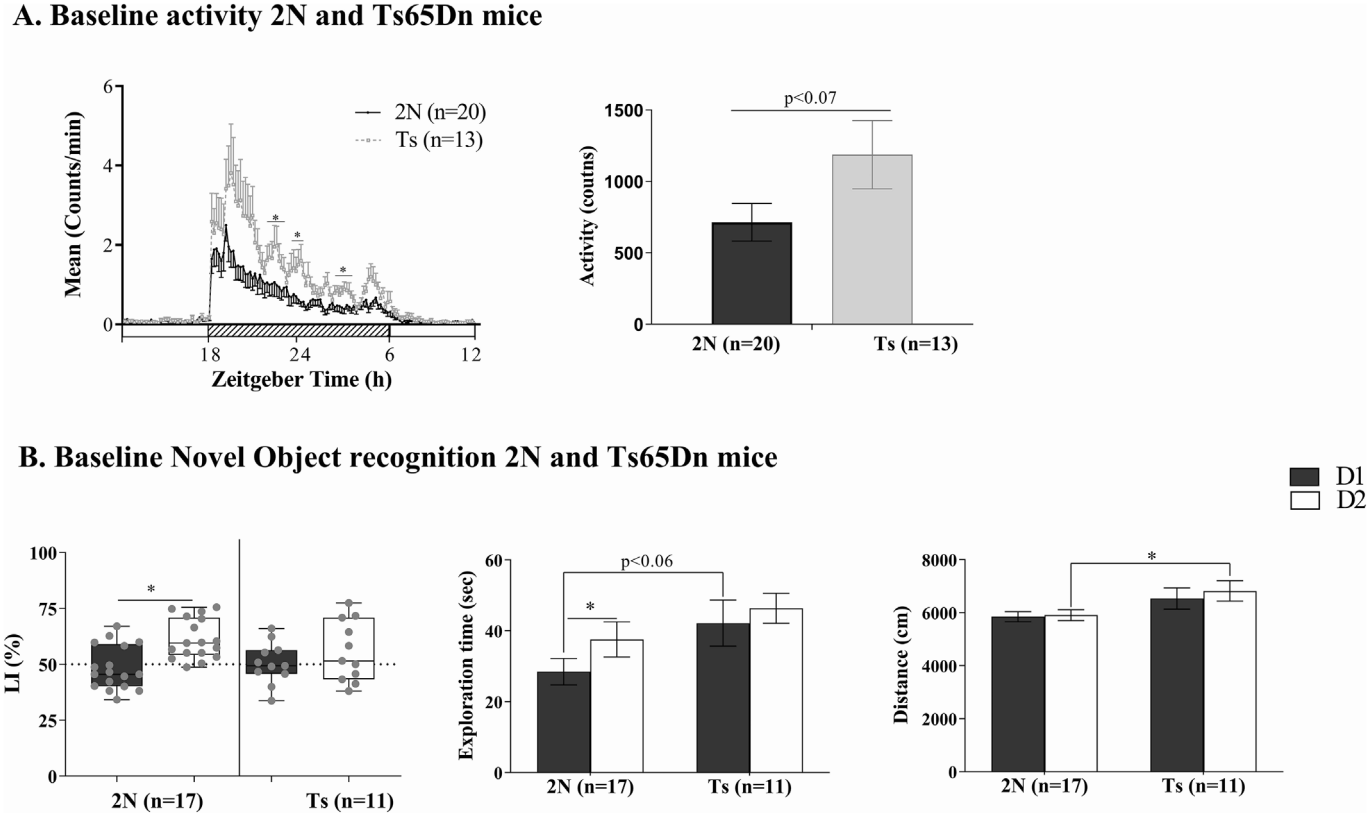

Fig. 2. (A) Profile of activity and (B) learning indices (LI) as well as exploration time and distance travelled during the Novel Object Recognition (NOR) for the 2N and the Ts65Dn (Ts) mice at baseline. Both 2N and Ts65Dn mice showed a light/dark activity pattern but the Ts65Dn mice are more active (*, $p < 0.05$). For the NOR, the training was done during day 1 (D1) and the testing during day 2 (D2). Data are plotted as box and whisker plots with median values and percentiles at 10 and 90%. Circles show data points for individual animals. Ts65Dn mice have memory impairment at baseline (*, $p < 0.05$) and travelled significantly more distance during the NOR testing than 2N (*, $p < 0.05$).

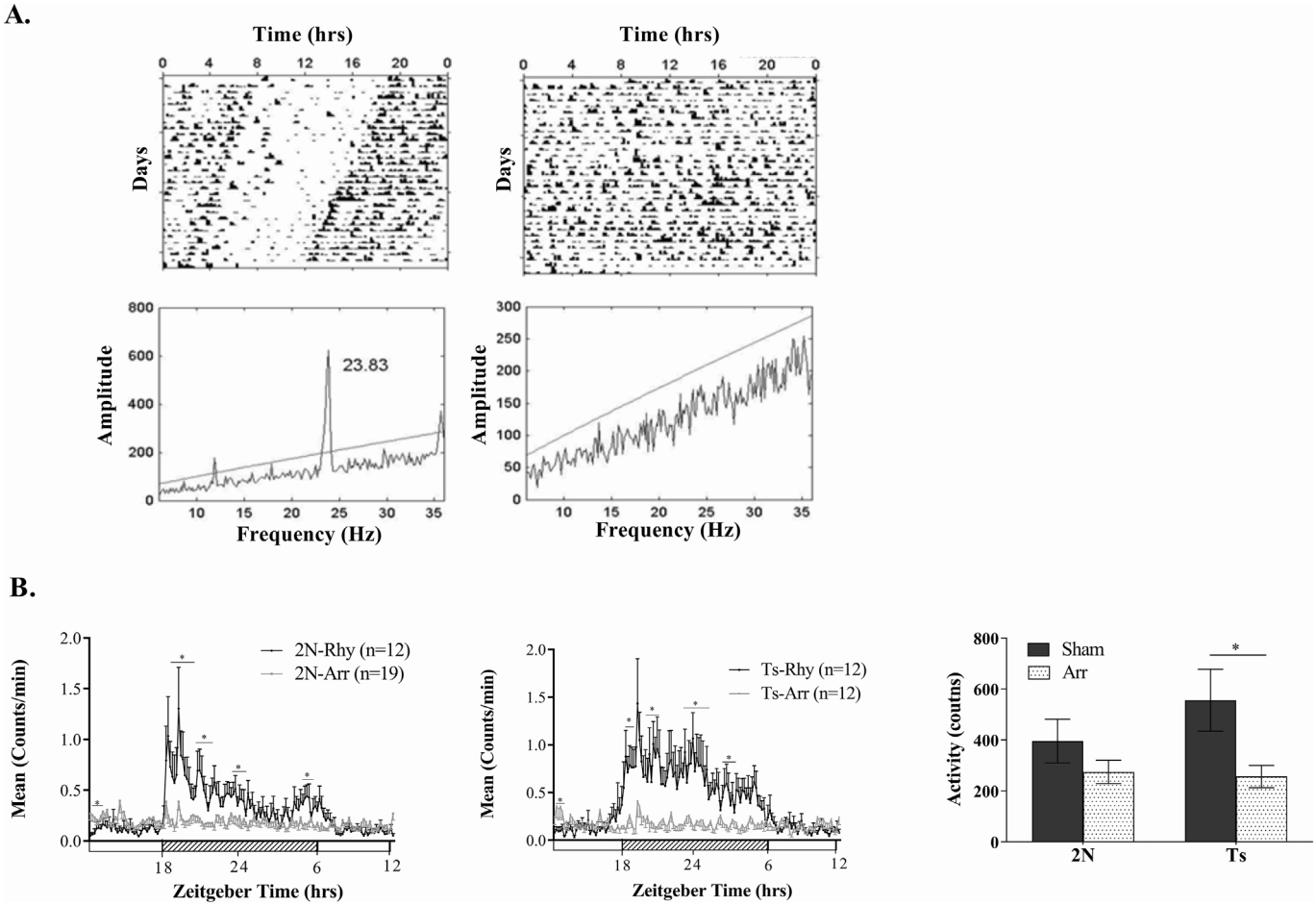

Fig. 3. Profile of activity of the 2N and Ts65Dn (Ts) mice that were arrhythmic (Arr) after surgery. (A) Example of actogram before the lesion (left) and after (right). The lesion led to a loss of rhythmicity. (B) Activity of 2N and Ts65Dn mice that were arrhythmic (Arr) or not (Rhy) after surgery (*, $p < 0.05$). A 24 h. pattern of activity is present only for rhythmic mice. Only the Ts65Dn mice showed significantly less activity after the lesion (*, $p < 0.05$).

learning and memory. In a pioneering study Stephan and Kovacevic (1978) lesioned the SCNs of rats rendering them circadian arrhythmic. They used the metric of passive avoidance of foot shock to assess memory at different circadian phases. They found that the lesioned animals had lost their daily oscillation of behavior in this memory task, but they were performing at the highest levels at all times rather than the lowest. In other words, memory seemed to be improved in the lesioned animals.

Another animal model of circadian involvement in learning disability is the Siberian hamster (*Phodopus sungorus*). These animals have robust circadian rhythms and typical phase response curves when exposed to a short light stimulus at different phases of their free-running rhythms. However, when these animals are held on a long day photoperiod and exposed to phase-advancing light stimulus on one night and then a phase-delaying stimulus on the next day, most of the animals become permanently circadian arrhythmic and do not respond to a light/dark cycle (Ruby et al., 1996). These arrhythmic hamsters have severe deficits in forming short- and long-term memories (Ruby et al., 2008). PTZ treatment, however, restores their learning abilities without restoring their circadian rhythms (Ruby et al., 2013). Another treatment that restores the learning ability to arrhythmic hamsters is lesion of their SCNs. Hamsters were made arrhythmic with the light treatment and shown to be learning disabled. They then received SCN lesions following which their learning abilities were restored, but only in those hamsters shown to have complete lesions (Fernandez et al., 2014). The obvious conclusion of these studies was that circadian arrhythmia per se does not impair learning and memory, but an arrhythmic SCN does.

The Ts65Dn mouse is not arrhythmic. To the contrary, it has robust circadian rhythms (Ruby et al., 2010). We therefore propose that both continuous SCN activity as in the hamster, or abnormally high SCN activity during the light phase in the Ts65Dn mouse impairs their abilities to consolidate memories. When the SCN are removed from both of these learning-disabled animals, neural plasticity and the ability to consolidate long-term memories is restored. Since the SCN are GABAergic nuclei, we propose that directly or indirectly the SCN of Ts65Dn mice are responsible for the over-inhibition that is responsible for their learning disability.

We showed here that complete lesion of the SCN improves learning and memory in a mouse model of Down syndrome. While the data presented here are consistent, they must be interpreted with caution as damage from the lesions was not restricted to just the SCN. The Ts65Dn mice in the CL group sustained extensive and variable damage to adjacent hypothalamic areas. Thus, the observed memory improvements cannot be attributed solely to the SCN based on these data. For example, these animals also experienced damage to the hypothalamic paraventricular nucleus which is critical for activation of the hypothalamic-pituitary-adrenal (HPA) axis. Thus, these animals would have been unable to secrete hormones involved in the stress response. Although all of our mice were handled extensively over many days prior to testing as a stress reduction measure, it is still possible that an inability to secrete stress hormones facilitated the improved performance of the Ts65n mice in the NOR test. Likewise, we cannot rule out the possibility that improved NOR performance could be attributed to other damaged brain areas. However, it is notable that partial SCN

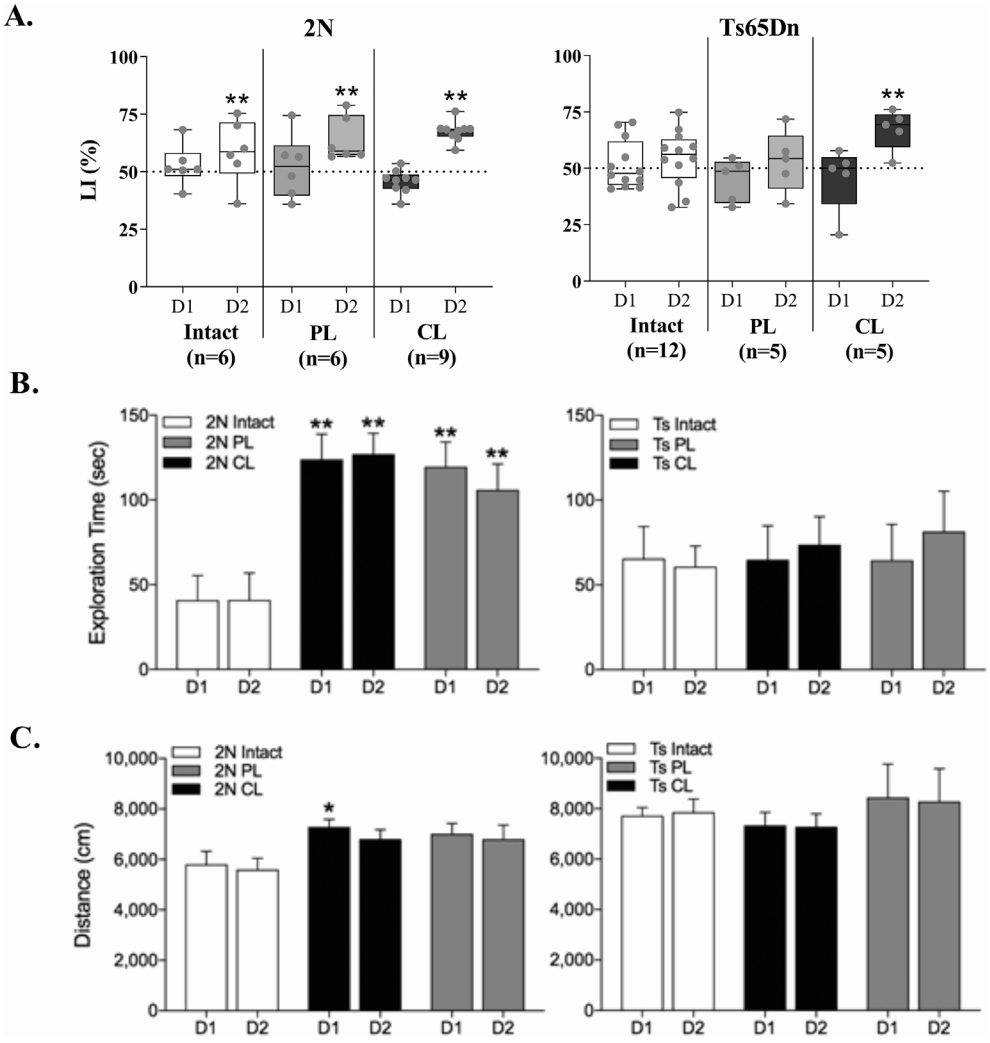

Fig. 4. (A) Learning Index (LI) scores in the NOR test for 2N controls (left) and for Ts65Dn (Ts, right) mice. Data are plotted as box and whisker plots with median values and percentiles at 10 and 90%. Circles show data points for individual animals. Scores are plotted for the training phase on day 1 (D1) and for the testing phase on day 2 (D2) for animals in which the SCN was intact, partially lesioned (PL), or completely lesioned (CL). The exploration times (B) and the total distance (C) travelled by mice during the NOR training (D1) and testing phase (D2) for 2N controls (left) and Ts65Dn mice (Ts, right) depending of the lesion of animals: SCN intact, partially lesioned (PL), or completely lesioned (CL; *p < 0.05).

lesions, in spite of the extensive damage to neighboring hypothalamic tissues, did not improve NOR performance. The concern about lack of specificity of the lesion methodology would be more serious if we were reporting on a loss of function, but in these studies, the remarkable result is a gain of function. Also, the Ts65Dn mice carry three copies of an extra segment with non-DS-related genes including protein-coding-genes, non-protein-coding-genes and pseudogenes (Herault et al., 2017) which could lead to potential problems with effects of these non-DS-related-genes on recognition memory. Therefore, it could be interesting to confirm our results by repeating this experiment on other DS model mice, like Ts1Cje, which show less memory deficits and have less triplicated genes than the Ts65Dn model mice (Fernandez and Garner, 2007; Sago et al., 1998).

Another significant corollary finding in this study is that the extensive brain damage experienced by the 2N control mice with either partial or complete SCN lesions did not impair their performance on the NOR test. Tissue damage in these animals was comparable to the Ts65Dn mice, and yet, their performance was not impaired. Apparently, damage to regulatory areas in the hypothalamus was not sufficient to interfere with hippocampal or cortical processing of recognition memory.

5. Conclusions

Complete lesion of the master circadian clock in the brain, the SCN, improves at least one type of learning and memory in a mouse model of Down syndrome. Improvement of an important brain function by removal of a part of the brain seems counter-intuitive. We do know that learning and memory show circadian variations. Our results suggest that the apparent decrement in neuroplasticity at certain circadian phases is controlled and is not simply due to fatigue or sleepiness. What could be the adaptive value of active suppression of neuroplasticity? We hypothesize that during wake, high levels of neuroplasticity are essential for the coding of new information into short-term memory. However, when those newly formed short-term memories are being consolidated during subsequent sleep, it is important to stabilize them to guarantee their fidelity. We propose that a previously unrecognized function of the circadian system is to stabilize short-term memory transcripts while they are being consolidated and transferred to long-term memory during sleep.

CRediT authorship contribution statement

Bayarsaikhan Chuluun: Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision. **Elsa Pittaras:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing - review & editing. **Hyunseung Hong:** Investigation. **Nathan Fisher:** Data curation, Investigation, Methodology, Visualization. **Damien Colas:** Conceptualization. **Norman F. Ruby:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - review & editing. **H. Craig Heller:** Conceptualization, Funding acquisition, Project administration, Writing - original draft.

Declaration of competing interest

We don't have any conflict of interest to declare. This work was supported by The LuMind Foundation, The Jerome LeJeune Foundation, The Matthew Foundation, and The Fyssen Foundation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbscr.2020.100049.

Abbreviations

Arr	arrhythmic
CL	completely lesioned SCN
DS	Down syndrome
D1	day 1
D2	day 2
LD	light/dark
LI	Learning Index
LTP	long-term potentiation
NOR	Novel Object Recognition
PL	partially lesioned SCN
PTZ	Pentylenetetrazole Ts: Ts65Dn
Rhy	Rhythmic
SCN	Suprachiasmatic nucleus
ZT	zeitgeber time

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