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NREM consolidation and increased spindle counts improve age-related memory impairments and hippocampal representations

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Keywords: NREM, spindles, place cells, sleep, aging, memory, remapping

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Abstract

Age-related changes in sleep patterns have been linked to cognitive decline. Specifically, age is associated with increased fragmentation of sleep and wake cycles. Yet it remains unknown if improvements in sleep architecture can ameliorate cellular and cognitive deficits. We evaluated how changes in sleep architecture following sleep restriction affected hippocampal representations and memory in young and old mice. Following training in a hippocampus-dependent object/place recognition task, control animals were allowed to sleep normally, while experimental animals underwent 5 hr of sleep restriction (SR). Interestingly, old SR mice exhibited proper object/place memory, similarly to young control mice, whereas young SR and old control mice did not. Successful memory correlated with the presence of two hippocampal cell types: 1) “Context” cells, which remained stable throughout training and testing, and 2) “Object” cells, which shifted their preferred firing location when objects were introduced to the context and moved during testing. As expected, EEG analysis revealed more fragmented sleep and fewer initial spindles in old controls than young controls during the post-training sleep period. However, following the acute SR session old animals displayed more consolidate NREM and increased spindle count, while young mice did not significantly display changes in sleep architecture. These results indicate that consolidation of NREM sleep and increases in spindle numbers serve to ameliorate age-related memory deficits and allow hippocampal representations to adapt to changing environments.

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Significance Statement

Age-related cognitive decline is associated with poor sleep quality. Interestingly, while sleep restriction has strong negative effects in young subjects, it does not affect or leads to performance improvements in old ones. This study investigated the possibility that sleep restriction differentially affected sleep architecture in young and old mice, leading to distinct cellular and cognitive phenotypes. In young animals, sleep restriction produced memory impairments and less flexible hippocampal representations, without significantly affecting sleep quality. However, in old animals, it led to improved sleep quality, enhanced memory, and more precise and flexible hippocampal representations. These findings have important implications because they indicate that the risk factors associated with poor sleep quality and age-related cognitive decline can be modifiable.

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Introduction

Mounting evidence suggests that sleep plays a key role in memory consolidation (Abel et al., 2013; Rasch and Born, 2013; Stickgold and Walker, 2013; Tononi and Cirelli, 2014). Studies have shown performance gains following post-training sleep (Smith, 2001; Gais and Born, 2004a, b; Gais et al., 2006), as well as learning impairments when sleep restriction (SR) is conducted after training (Smith and Rose, 1996; Graves et al., 2003; Prince et al., 2014). However, the effects of SR appear to vary across the lifespan and are more complex than it appears. For example, partial SR has minimal effects on adolescent cognitive performance (de Bruin et al., 2017) and sleep loss can differentially impact adults (Krause et al., 2017), with some studies showing within- and across-individual differences in cognitive susceptibility (Saletin et al., 2016; Wilson et al., 2019). Interestingly, SR therapy, which is characterized by limiting sleep periods, has very positive effects on the sleep quality of old subjects (Wennberg et al., 2013), yet the underlying physiological and cellular changes associated with this improvement remain unclear.

Sleep involves intercalated periods of non-rapid eye movement (NREM), a state characterized by high amplitude, low frequency (0.2-4 Hz), synchronous electroencephalographic (EEG) activity, with periods of rapid eye movement (REM), characterized by low amplitude fast desynchronized EEG waves. It has been proposed that NREM is particularly important for memory formation and transfer of information to cortex (for a review see, (Antony et al., 2019), whereas REM has been associated with both consolidation of novel information and forgetting of previously encoded information (Poe, 2017). During NREM, there are rapid bursts of activity (10-14 Hz) of short duration, known as spindles. It is thought that spindles facilitate memory reactivation, which is essential for proper consolidation (Rasch and Born, 2013). Critically, changes in NREM and spindle characteristics predict early memory

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impairments in older subjects (Taillard et al., 2019). However, it remains unknown if reversing spindle changes can have positive effects on cognition.

The hippocampus plays a critical role in the formation of episodic memories – recollections of events happening in specific contexts at particular times (Squire and Zola, 1998; Smith and Mizumori, 2006). The activity of hippocampal place cells, which fire in specific locations when animals navigate (O'Keefe and Dostrovsky, 1971), provides a cognitive map in which episodic memories are embedded (Mizumori, 2006; Smith and Mizumori, 2006). Evidence supporting this idea comes from the observation that networks of cells active during wake are reactivated during sleep at a compressed time scales during NREM (Lee and Wilson, 2002; Drieu et al., 2018; Hwaun and Colgin, 2019), which has been shown to be important for goal-directed memories (de Lavilleon et al., 2015).

Both sleep and cognition have been shown to undergo age-related changes across the lifespan (Huang et al., 2002). Older humans exhibit more fragmented sleep and less slow wave sleep in comparison to young adults (Ohayon et al., 2004; Espiritu, 2008; Hasan et al., 2012). Additionally, older subjects exhibit impairments in hippocampus-dependent cognitive tasks (Miller and O'Callaghan, 2005; Lister and Barnes, 2009; Lester et al., 2017). These observations have suggested that age-related cognitive decline may be linked to changes in sleep patterns (Altena et al., 2010); however, the relationship between sleep quality and hippocampal activity during wake periods remains unclear. Here, we investigated if an acute period of SR could modify subsequent sleep architecture, hippocampal place cell firing, and object-place recognition (OPR) memory in young and old mice. Our results indicate that SR has differential effects in young and old mice. In young mice, an acute SR session does not significantly affect sleep architecture but it decreases the flexibility of hippocampal representations and memory. Conversely, in old animals, SR leads to NREM consolidation and

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increases in spindle count, which allows the hippocampus to adapt to changing environments and reverses age-related memory deficits.

Materials and Methods

Subjects

Young (8-24 weeks old) and aged (54-72 weeks old) adult male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were housed individually on a 12-hour light/dark cycle and allowed access to food and water *ad libitum*. Animal living conditions were consistent with the standard required by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experiments were approved by the Institution of Animal Care and Use Committee of the University of Texas at San Antonio and were carried out in accordance with NIH guidelines.

Surgery

For sleep recordings, prefabricated 2 channel EEG/1 channel EMG headmounts (Pinnacle Technology) were implanted [from Bregma (in mm): frontal leads: AP: +3.2mm, ML: ±1.2mm, and parietal leads: AP: -1.8mm, ML: ±1.2mm] and secured with cyanoacrylate and dental cement. Two EMG leads were placed under the nuchal musculature and affixed with VetBond. For place cell recordings, drivable 6-tetrode headstages were affixed to the skull with cyanoacrylate and dental cement, with recording electrodes placed directly above the dorsal hippocampus [from Bregma (in mm): AP, -1.7; ML, -1.6; form dura; DV, -1.0] (Wang et al., 2012; Wang et al., 2015)). Sleep patterns were not analyzed in mice implanted with tetrodes because the EMG lead made the tetrode implants unstable and noisy. Animals underwent at least one week of recovery prior to recordings.

Sleep Restriction (SR)

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An automated SR cylindrical apparatus (Pinnacle Technology, Lawrence, KS) containing a bar spanning the enclosure was used for all SR procedures. Animals were individually housed in the apparatus starting at least 24 hours prior to the beginning of the experiments with fresh bedding, food, and water, and were returned to the apparatus in between trials. To induce SR, the bar was rotated continuously by a motor at approximately 3 rpm with random reversals in direction to prevent that animals sleep during brief periods by predicting the pattern of rotation. All SR procedures followed the object exposures and therefore, were started within 4 hr after the lights were on.

Experimental Design and Statistical Analysis

Behavioral Training

For all animals, behavioral procedures (e.g., exposure to the objects on day 1 and memory testing on day 2) were conducted during the first 4 hr of the light cycle (ZT 0-4). The object/place recognition task (OPR) was conducted in a square context (30cm x 30cm) with visual features on each wall for orientation (Figure 1A). Everyday items (glass beer bottle, metal soda can, and plastic juice bottle) were used as objects after pilot testing determined mice showed roughly equal preference for all items on average. On day 1, animals were habituated to the empty context for a habituation trial (Hab). After the habituation, the 3 objects were arranged along one of the diagonal axes of the context and 3 object exploration trials (T1-3) were conducted. During the inter-trial interval (2 min), the context and objects were wiped down with 70% ethanol. Immediately following the third object trial (T3), animals in the experimental groups were housed in the experimental chamber and were sleep deprived for 5 hr, whereas controls were housed in the same chamber but allowed to sleep. On day 2, one object was moved from its original location to an adjacent corner and mice were tested in the context with the moved object (Test). All trials were 6 min in duration. Object positions were counterbalanced across trials.

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Behavioral Analysis

All object exploration trials were video recorded using Limelight (Actimetrics, Wilmette, IL) and analyzed offline by researchers blind to the group condition. All instances when an animal was oriented toward and touching an object with nose, vibrissae, and/or forelegs were coded as "object exploration"; contacting an object while passing or oriented away were not considered. Animals with an average object exploration time less than 10 seconds on any trial were excluded from analysis. Behavioral data from animals used for sleep analysis was combined with data from animals used for place cell recordings.

Object preference was calculated as the percentage of time spent exploring the moved object relative to total object exploration time during testing minus the relative time exploring the same object during training, as previously described (Oliveira et al., 2010). This method estimated object preference taking into account any potential bias that animals may have had during training. Specifically, percent change in preference was calculated using the following formula:

$$\%\ change\ in\ preference = 100 * \left(\frac{moved\ object\ exploration_{test}}{total\ object\ exploration_{test}} - \frac{moved\ object\ exploration_{trial\ 2-4}}{total\ object\ exploration_{trial\ 2-4}}\right)$$

Larger percent change indicates greater preference for the moved object during the test session, while lower values indicate little change in preference from day 1 to day 2, after the object is displaced. To rule out that novelty effects recorded on Day 1, we repeated the same analysis excluding trial 1 when the objects were first introduced, obtaining almost identical results (Figure 1S).

Place Cell Recordings and Analysis

Beginning one week after surgery, neural activity was screened daily in an environment different from the context used for experiments, advancing the electrode bundle 15-20 μm per

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day until pyramidal cells could be identified by their characteristic firing patterns (Ranck, 1973). Lowering the electrodes in small steps minimizes electrode drift and ensures recording stability for several days (Muzzio et al., 2009b; Wang et al., 2012). Moreover, all animals yielding unit data remained connected to the recoding setup via a commutator throughout the experiment to further minimize the possibility of electrode drift during the plugging/unplugging. Long-term recordings were considered stable when cells had the same cluster boundaries over two sessions (at least 24 hr apart), and the waveforms obtained from all four wires of a tetrode were identical. Experiments began only when recordings were stable for 24 hr. Animal position and electrophysiological data were recorded using Cheetah Data Acquisition system (Neuralynx, Bozeman, MN), as previously described (Wang et al., 2012; Wang et al., 2015).

Units were isolated using MClust software (developed by A. David Redish, University of Minnesota) and accepted for analysis only if they formed isolated clusters with clear Gaussian ellipses and minimal overlap with surrounding cells and noise. All cells were inspected to rule out the presence of events during the 2 msec refractory period. Place field maps were generated using custom Matlab code as previously described (Wang et al., 2012; Keinath et al., 2014; Wang et al., 2015). Briefly, the arena was first divided into a 70x70 pixel grid and an activity map (the total number of spikes in each pixel), and a sampling map (the total amount of time spent in each pixel) were computed. Both maps were then smoothed with a 3 cm standard deviation Gaussian kernel. The activity map was then divided by the sampling map, which yielded the place field map. Any location sampled for less than 1 s was considered un-sampled. Only periods of movement were included in the analysis (minimum walking speed: 2 cm/s). Cells that fired less than 25 spikes during movement or displayed peak firing frequencies below 1 Hz before smoothing were excluded from analysis. Firing rate patterns were characterized by computing the overall mean (total number of spikes divided by time spent in the arena), peak (maximum), and out of field (spikes occurring outside areas defined as place fields) firing rates. Place fields were defined as any set of at least 9 contiguous pixels in which the average firing

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rate was at least 20% of the peak firing rate (Rowland et al., 2011). If a cell yielded multiple place fields, the average of all fields was taken as the place field size. Rate remapping was calculated as the absolute difference between the peak firing rate of individual cells on consecutive trials. The spatial information content, a parameter that estimates how well the firing pattern of a given cell predicts the location of the animal, was computed as previously described (Skaggs et al., 1993) using the following formula $IC=\Sigma pi(Ri/R)\log(Ri/R)$,where pi is the probability of occupying location i, Ri is the firing rate at location i, and R is the overall mean firing rate.

Place field stability was assessed by calculating pixel-to-pixel cross-correlations between maps. The generated Pearson R correlation value reflected the degree of map similarity across trials for all cells. Overall global remapping was estimated by averaging the Pearson r correlation values across cells and animals in each condition. Additionally, cell types were classified into three categories depending on whether they remapped in the presence of the objects (object cells), remained stable throughout training (context cells), or displayed both short- and long-term instability (unstable cells), with stability defined as a correlation value above 0.4, a threshold previously used in mice (Muzzio et al., 2009b; Wang et al., 2012).

Verification of electrode placement

Tetrode placements were verified after completion of the experiments by passing a small current (0.1 mA) for 5 seconds through the tetrodes that yielded data in anesthetized animals. The brains were removed and fixed in 10% formalin containing 3% potassium ferrocyanide for 24 hr. The tissue was cryosectioned and stained using standard histological procedures (Powers and Clark, 1955).

Sleep State Analysis

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EEG/EMG signals were recorded for 10 hours following training on day 1 (Pinnacle Technology, Lawrence, KS). The headmounts were attached to a preamplifier for first stage amplification (100x) and initial high-pass filtering (0.5 Hz for EEG and 10 Hz for EMG). All signals were then sampled at 300 Hz and digitized (Sirenia Acquisition software, Pinnacle Technology). Animals with excessive noise in any channels (>10% of epochs classified as artifact) were discarded from analysis (4 animals were excluded from the post-training session due to noise).

Sleep recordings were divided into 4 sec epochs. 5% of epochs were randomly selected for manual scoring with Sirenia Sleep analysis software using EMG power and EEG amplitude and frequency to categorize REM, NREM, and WAKE states. Full and scored EEG/EMG files were then exported to MATLAB and the remaining epochs were analyzed using a naïve Bayesian classifier, a highly accurate method demonstrated to produce inter-rater agreements of 92% (Rytkonen et al., 2011).

To analyze delta and theta power we used the periods of NREM and REM extracted from the respective Bayesian analysis. The power spectral density (PSR) was estimated using Welch's method with a window size of 8 sec for delta and 0.4 sec for theta. Total power was computed from the post-spectral density using trapezoidal numerical integration. Relative power was then computed as the power in a given frequency band divided by the total power over all frequencies. Delta frequency: 0.25-4 Hz, theta frequency: 4-10 Hz.

Spindle detection during NREM was computed using a validated automated system for rapid and reliable detection of spindles using mouse EEG. This method eliminates observer bias and allows quantification of sleep parameters including count, duration, and frequency as well as rapid quantification during selective sleep segments (code generously provided by Dr. David Uygun, for analysis details see (Uygun et al., 2019).

Code accessibility: All code and analysis tools will be available upon request.

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Statistics

Statistical analysis was performed with SigmaStat (Ashburn, VA). One way ANOVA was used to compare object preference during the test session, number and length of sleep bouts as well as percent time in wake, NREM, and REM. T-tests were used to compare sleep variables prior to sleep restriction, Two-way ANOVAs with repeated measures were used to compare place cell correlations across sessions, rate remapping, all parameters to evaluate place cell characteristics, and differences in spindle count or properties on repeated segments during sleep. One-way ANOVAs were used to calculate differences in delta and theta relative power and spindles characteristics (count, duration, and frequency) across groups. Student-Newman Keuls (SNK) multiple comparisons were used to determine which groups were significantly different. A significance level of 0.05 was used for all tests.

Results

Sleep restriction impairs object-place recognition memory in young adult mice but enhances performance in old mice

All animals included in this study were maintained on a 12/12 light/dark (L/D) cycle and were trained during the first 4 hr of the light cycle. Object preference was assessed following training and testing in the OPR task (Figure **1A**). The results indicated that the groups displayed differential object/place recognition [effect of group on object preference: $F_{(3,62)}=4.31$, $p<0.009$]. SNKs multiple comparisons demonstrated that young control and old SR mice displayed significantly greater preference for the moved object ($p<0.05$) in comparison to young SR or old control mice (Figure **1B**). Moreover, object preference in old SR mice was comparable to that observed in young control animals ($p>0.05$).

We also found no significant group or interaction effects on total object exploration times across trials [main effect of group: $F_{(3,56)}=0.07$, $p=0.97$; interaction: [$F_{(9,168)}=0.41$, $p=0.93$, Figure **1C**]. However, all groups displayed higher exploration during the first object trial (T1)

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[$F_{(3,168)}$=15.60,p< 0.001; T1<0.05], which likely resulted as a consequence of the novelty of the objects (Figure **1C**). Since we observed this difference, we also calculated the object preference excluding the first object trial to ensure that our results were not biased by a novelty effect. The results were almost identical to those including all trials (percent change in preference: young control: 14.30±3.43, young SR: -0.01±4.56, old control: -0.80±5.07, old SR: 19.73±3.51, Figure 1S). These findings demonstrated that the differences in object preference observed across the groups were not due to variability in object exploration.

Rate remapping increases during testing in young control and old sleep deprived animals

A subset of animals was implanted with tetrodes in area CA1 to determine the effects of sleep restriction on hippocampal cell firing (electrode positions are shown in Figure **2A**). We examined firing rate changes in 110 cells recorded in 10 young mice (60 cells in 6 controls and 50 cells in 4 SR mice) and 79 cells recorded in 10 old mice (42 cells in 5 controls and 37 cells in 5 SR mice). There were no significant effects of group or group by trial interactions in mean, peak, or out of field firing rate (p>0.05, Figure **2B-D**). However, there was an effect of trial [mean firing rate: $F_{(4,739)}$=5.57, p<0.001; peak firing rate: $F_{(4,739)}$=3.45, p<0.009; out of field firing rate: $F_{(4,739)}$=5.42, p<0.001]. SNKs post hoc tests indicated that animals displayed higher mean and out of field firing rates during object trials 1 and 2 and higher peak firing rate in trial 2 than the habituation and test trials (p<0.05, Figures **2B-D**). However, there were no significant differences in firing rate across the object trials (p>0.05). These data suggest that the exposure to the objects increases overall firing rate, but there were no significant differences across the group conditions throughout training and testing.

Hippocampal cells have previously been shown to code environmental changes through increases or decreases in firing activity, a phenomenon known as rate remapping (Leutgeb et al., 2005). Therefore, we hypothesized that the analysis of average activity could have masked potential rate differences. To assess this possibility, we calculated rate remapping as the

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absolute difference in peak firing rate for each cell across trials. This analysis revealed a trend in the main effect of group [$F_{(3,185)}$=2.30, p=0.08], a significant effect of trial [$F_{(3,554)}$=8.66, p<0.001] and a significant group by trial interaction [$F_{(9,554)}$=2.32, p<0.02]. SNKs multiple comparisons indicated that there were significant differences in rate remapping between the last training trial (T3) and the retrieval trial (Test). Rate remapping was highest in young controls and old SR animals, the two groups that exhibited successful learning (p<0.05, Figure **2E**). These results indicate that changes in rate remapping are important for updating object memory representations in the OPR task.

An acute session of sleep restriction increases the spatial information content of hippocampal cells in old mice, but has no effect in young mice

We next examined the spatial information content of the cells, a parameter that determines how well a cell predicts the location of an animal (Skaggs et al., 1993). We found an effect of trial and an interaction between trial and group [effect of trial: $F_{(4,739)}$= 6.88, p<0.001; interaction: $F_{(12, 739)}$=2.41, p<0.006]. SNKs multiple comparisons showed that cells from old SR animals displayed more spatial information content than cells from old controls on the Test trial (p<0.02). However, no differences were observed in the young groups (p>0.05, Figure **3A**). Finally, there were no differences across the groups, trials, or interactions in average field size or number of fields (p>0.05, Figure 2S). These results indicate that the spatial properties of hippocampal cells from old mice improve after an acute session of sleep restriction, while other parameters remain constant.

The overall stability of hippocampal representations decreases during the moved-object test in all groups

Previous studies have shown that dorsal hippocampal cells respond to objects (Cohen et al., 2013). Therefore, we expected that cells would shift their preferred firing locations (i.e.

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global remapping) when the objects were first introduced (Hab vs. T1), but display short-term stability across the training trials while the objects and environment remained unchanged (T1 to T3). We also anticipated global remapping during the test trial (Test), reflecting that animals perceived the change in the object configuration or that cells became unstable in the long term, as previously reported in mice (Muzzio et al., 2009a; Muzzio et al., 2009b). Analysis of similarity between the place cell maps revealed a significant effect of group [$F_{(3,185)}$=3.77, $p<0.02$], trial [$F_{(3,554)}$=56.48, $p<0.001$], and interaction [$F_{(9,554)}$=2.58,$p<0.007$]. SNKs multiple comparisons revealed that old animals (control and SR groups) displayed more remapping than young animals (control and SR groups), when the objects were first introduced (Hab/T1, $p<0.05$). This likely reflected the more pronounced instability in spatial representations previously reported in old animals (Barnes et al., 1997). However, no differences in stability were observed during the object training trials (T1-3, $p>0.05$). As expected, all groups displayed lower place field stability during the test trial in comparison to the high short-term stability observed during training ($p<0.05$), with old control animals showing more instability than all the other groups ($p<0.05$, Figure **3B**). These data indicated that all groups exhibit some global remapping during the moved object test trial.

Cell types responding to distinct aspects of the environment are differentially affected by sleep restriction and age

We have previously observed that distinct CA1 neurons display different behavioral phenotypes during fear learning (Wang et al., 2015). To determine if different subpopulations of cells were responding to distinct aspects of the environment in the OPR task, cells were classified according to their remapping patterns during training. “Context” cells displayed high stability throughout habituation and training; “object” cells remapped when objects were first introduced but remained stable during all the object training sessions; and “unstable” cells remapped across all training sessions. In young animals, each of these categories made up

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roughly 1/3 of the cells recorded (Figure **3C**). However, old animals had a lower percentage of stable context cells than young mice (Figure **3D**).

After cells were classified, we examined remapping within each group across trials (Figure **4A**). We hypothesized that if animals remembered the environment, context cells should remain stable during the moved-object test. However, this was not the case in all groups. Context cells showed an effect of trial [$F_{(3,159)}$=27.55, $p<0.001$], and interaction [$F(_{9,159})$=2.14, $p<0.03$]. SNKs multiple comparisons indicated that context cells in old control animals were more unstable than in all the other groups ($p<0.05$, Figure **4B**}. Interestingly, there was no difference between young controls and young SR mice in the stability of context cells ($p>0.05$), suggesting that the memory impairment observed in the latter was not due to a failure recalling the environment.

We also hypothesized that if animals noticed the change in the object configuration, object cells should display remapping during the test. However, if animals failed to notice the moved object, these cells should remain stable. Object cells displayed an effect of group [$F_{(3,60)}$=4.77, $p<0.006$] and trial [$F_{(3,177)}$=120.14, $p<0.001$], as well as an interaction between these variables [$F_{(9,177)}$ =2.26, $p<0.03$, Figure **4C**]. SNKs multiple comparisons revealed that there were no differences in objects cells during training across the groups ($p>0.05$). However, object cells in young controls and old SR mice displayed significant remapping in comparison to those in young SR mice during the test trial ($p<0.05$). These results indicate that object cells respond to the displacement of the object in animals that show proper memory. Object cells from old controls also remapped during the test trial; however, this likely reflected an overall lack of long-term stability in this group. Finally, there were no stability differences in “unstable” cells across groups or trials [group: $F_{(3,60)}$=0.54, $p>0.05$; trial: $F_{(3,180)}$=1.5, $p>0.05$; interaction: ($F_{(9,180)}$=0.37, $p>0.05$; Figure **4D**]. Together, these data indicate that the proper memory performance observed in young controls and old SR mice correlates with stable context cells and flexible object cells.

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Old control animals display fragmented sleep patterns during post-lar4ning sleep in comparison to young animals

Young and old control animals with EEG/EMG microdrives were placed in a SR chamber (Figure **5A**), while *ad lib* sleep was recorded and analyzed for 5 hr following training (Figure **5B**). We found that the total percent time spent in wake, NREM and REM was equivalent in young and old control animals ($p>0.05$, Figure **5C**). However, old animals displayed significantly more wake [$t_{(18)}= 2.27$, $p<0.04$] and NREM bouts [$t(_{(18)}= 2.59$, $p<0.02$] as well as a trend toward more REM bouts [$t(18)= 2.00$, $p=0.09$] than young controls (Figure **5D**). Additionally, old mice displayed shorter wake bout length [$t_{(18)}= 2.28$, $p<0.04$] and a trend toward shorter NREM bout length [$t_{(18)}=)= 1.95$, $p=0.067$] than young controls (Figure **5E)**, indicating that old animals displayed more fragmented sleep than young mice, as previously observed (Pace-Schott and Spencer, 2015).

Old mice do not exhibit a sleep rebound after restriction, but show increased NREM consolidation

Following the initial 5 hr post-training period, we recorded 5 additional hours of *ad lib* sleep in the control and SR groups to evaluate the effects of SR on recovery sleep. Old animals did not display differences in total time spent in wake or sleep states. Moreover, although on average young SR animals displayed less wake and more NREM than control mice, the differences were not significant [Figure **5F**, wake: $F_{(3,35)}= 1.62$, $p=0.08$, NREM: $F_{(3,35)}= 1.65$, $p=0.19$], REM: $F_{(3,35)}= 0.78$, $p=0.51$]. However, there were significant changes in sleep macrostructure. We observed differences in the number of NREM bouts [$F_{(3,35)}= 3.04$, $p<0.05$]. SNKs post-hoc tests indicated that the old SR mice had less number of bouts than the young controls ($p<0.05$), and displayed a trend toward less bouts in comparison to the young SR mice ($p=0.07$) and the old controls ($p=0.09$) (Figure **5G)**. Additionally, there were significant

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differences in NREM bout length [$F_{(3,35)}$=3.91,p<0.02]. Multiple comparisons indicated that old SR mice displayed significantly longer NREM bouts compared to old controls (p<0.03) and young SR mice displayed a trend toward longer bouts than young controls (p=0.09, Figure **5H**). The fact that old SR mice displayed less number of NREM bouts of longer length than the controls indicated that NREM was consolidated in this group. Finally, there were no differences across the groups in bout number or length during wake periods or REM periods (p>0.05, Figure **5G-H**). In summary, these data indicate that an acute session of SR affects sleep macrostructure in old animals, leading to NREM consolidation.

An acute sleep restriction session increases relative delta power (RDP) during NREM in young mice and relative theta power (RTP) during REM in young mice.

Delta power has been shown to increase following sleep deprivation ((Davis et al., 2011)). However, this effect depends on the duration of wakefulness (Halassa et al., 2009; Dispersyn et al., 2017), animal housing conditions (Kaushal et al., 2012), and several other variables (Davis et al., 2011). To determine if the experimental conditions of this study affected delta oscillations, we calculated RDP during NREM sleep across the groups. First, we assessed DRP during *ad lib* sleep following training, but found no significant differences between young and old control mice [$t_{(14)}$=0.03, p>0.05, Figure **6A**]. However, there were differences in RDP during the recovery period [($F_{(3,35)}$=4.11, p<0.02)]. SNKs multiple comparisons indicated that the young SR animals displayed more RDP than the young controls (p<0.04); but, there were no significant differences between the old groups (p>0.05; Figure **6B**). These results coincide with other reports showing increases in delta power in young animals following sleep deprivation (Halassa et al., 2009).

Theta oscillations during REM have also been associated with memory encoding (Hasselmo, 2006; Hutchison and Rathore, 2015). Therefore, we examined RTP during REM when this oscillation is most prominent. We did not observe significant differences during the

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post-learning *ad lib* sleep period [$t_{(14)=}$-1.3, p=0.21, Figure **6C**], but there were significant differences during recovery sleep [$F_{(3,35)}$=3.90, p<0.02). SNKs multiple comparisons indicated that young SR mice displayed lower RTP than all the other groups (p<0.05, Figure **6D**].

Young control mice display more spindles of longer length than old control mice immediately following training

Using a validated automated spindle detection method (Uygun et al., 2019), we then quantified number of spindles, duration, and frequency following training. There were no significant differences in number, duration, or frequency of spindles between young and old control mice during the *ad lib* post-training sleep period (p>0.05, Figures **7A-B** and **4SA**). Since the initial hours following training have been shown to be important for consolidation in young animals (Bailey et al., 2004), we divided the post-training sleep period into 1 hr segments to determine if spindles differ across time. We found that there was a significant effect of segment and interaction between group and segment [effect of segment: $F_{(4,48)}$=4.67, p<0.003; interaction: $F_{(4,48)}$=2.69, p<0.05]. SNKs multiple comparisons indicated that young control mice displayed more spindles in the first hour post-training (segment 1) in comparison to the old controls (p<0.05), with no significant differences between the groups after that (Figure **7C**). Similarly, analysis of spindle duration revealed a significant effect of session and interaction [effect of session: $F_{(4,48)}$=5.81, p<0.001; interaction of group and session: $F_{(4,48)}$=2.59, p<0.05]. SNKs post hoc tests indicated that young control mice displayed longer spindles during the first hour post-training in comparison to old control mice (p<0.05), but no significant differences after that (Figure **7D**). There were no differences in spindle frequency between the groups or interaction between segment and group condition (p>0.05, Figure 3S). These results indicate that in young control animals there are more spindles of longer duration immediately following training, which may contribute to consolidate memories more effectively in this group.

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An acute session of sleep restriction increases total number of spindles in old animals during recovery

Previous research has indicated that the time window for memory consolidation is much longer in old animals than young subjects (Schimanski and Barnes, 2010). These findings suggest that increases in spindle count occurring even several hours following learning may have a significant impact on memory consolidation in old animals. To address this possibility, spindle count, duration, and frequency were also examined during the recovery *ad lib* sleep period. We found that the average number of spindles were different across the groups [$F_{(3,35)}$=3.86, $p<0.03$, Figure **7E**]. SNKs multiple comparisons indicated that Old SR mice displayed more average number of spindles than old controls ($p<0.05$) and young SR mice ($p<0.05$) during the recovery period. No differences in spindle duration or frequency were observed across the groups ($p>0.05$; Figures **7F** and **4SC**).

We then examined if there were differences in spindle characteristics across the groups at different times during recovery by subdividing this period into 1 hr segments. We found a significant effect of group and segment on spindle count, but no interaction [group: $F_{(3,116)}$=3.73, $p<0.03$; segment: $F_{(4,116)}$=10.72, $p<0.0001$, Figure **7G**]. SNKs multiple comparisons indicated that the old SD group displayed more spindles than the old controls across all segments ($p<0.05$) and all groups displayed more spindles during the first two hours than during the last three segments of the recovery ($p<0.05$). There were no significant group, segment or interaction differences in spindle duration ($p>0.05$, Figure **7H**). Together, these results indicate that during recovery there is an increase in average spindle count in old SR mice in comparison to old controls, which may serve to consolidate memory and facilitate performance of the task.

Discussion

In this study, sleep restriction impaired object-place memory in young adult mice, but unexpectedly enhanced performance in old mice. The improved performance observed in old

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SR animals was accompanied by decreased NREM fragmentation and increased number of spindles during recovery sleep. Successful object-place recognition in both young control and old SR mice correlated with stability of the cells that coded the context and remapping of the cells that coded the configuration of the objects. Moreover, while both old control and young SR mice failed to recognize the displaced object, they exhibited different patterns of stability during the test session, indicating that performance deficits in these groups stemmed from distinct memory impairments. These data indicated that age-related cognitive deficiencies could be rescued by improving sleep architecture, which improves the flexibility and stability of hippocampal representations.

The unexpected finding that OPR memory was enhanced in old SR mice suggests that SR followed by a prolonged period of recovery sleep has mnemonic advantages. Sleep fragmentation has been associated with impaired memory consolidation (Tartar et al., 2006; Ward et al., 2009a; Ward et al., 2009b; Sportiche et al., 2010); thus, it is likely that NREM consolidation underlies the rescue of age-related cognitive deficits. Indeed, several studies have found that enhancing NREM sleep via pharmacological interventions or sleep restriction can provide protective effects on cognitive impairment following stroke or traumatic brain injury (Martinez-Vargas et al., 2012; Cam et al., 2013; Morawska et al., 2016). Further, changes in NREM activity predict memory performance (Ognjanovski et al., 2014; Ognjanovski et al., 2017) and disruptions of hippocampal oscillations during NREM sleep disrupt memory consolidation (Ognjanovski et al., 2018). Together, our findings and these data indicate that NREM is important for memory consolidation and enhancing its quality improves cognitive processes.

It is important to note that in our study, place cell instability in young control and old SR animals originated from object and unstable cells, while context cells remained highly stable even in the long term. Recent findings in mice found that hippocampal cells expressing cfos, a marker of activity that has been associated with the formation of memory engrams (Liu et al., 2014), are much more unstable than cells that do not express this early gene (Tanaka et al.,

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2018), confirming that subsets of cells participating in memory processes are indeed unstable. Moreover, it was shown that unstable subpopulations coexist with stable ones, potentially having distinct mnemonic functions. These observations are in line with our finding that the instability of "object" cells is important for memory updating.

Previous studies examining place cell activity during the OPR task, or variations of this task, have been conducted in young adult rats under normal sleep conditions (Zheng et al., 2016). For example, Larkin et al. showed that CA1 neurons exhibited changes in firing rate, but not in the cells' preferred firing locations during the moved object test (Larkin et al., 2014). Similarly, we observed rate remapping between the last object exploration trial and the test session in young control and old SR mice, suggesting that rate changes in both rats and mice are associated with object-place recognition. However, we also found that successful learning correlated with place field remapping in a subset of CA1 neurons during the moved-object test. The differences in stability between our observations and Larkin et al. may be related to the intrinsic stability differences between mice and rats (Kentros et al., 2004; Muzzio et al., 2009b), or to the different retention intervals used in these studies (5 min in Larkin et al. compared to $\geq$15 hr in our study).

The general consensus from other studies using the OPR task in young animals is that immediate post-training sleep is critical for memory. For example, performance was reported to be optimal when the retention interval occurred during the inactive phase of the light/dark cycle and rats were permitted to sleep (Binder et al., 2012). Furthermore, post-training SR has consistently shown to impair object-location memory in young adult mice (Havekes et al., 2014; Prince et al., 2014). Although these behavioral deficits have been attributed to disrupted memory consolidation, our results suggested otherwise. The long-term stability of "context" cells in young SR mice indicated that spatial representations formed during training were retrieved correctly, whereas the stability of "object" cells suggested that these animals failed to recognize the change in the object configuration. Therefore, while contextual memories remained intact,

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SR prevented recognition of specific changes in the environment. The synaptic homeostasis hypothesis proposed by Tononi and colleagues posits that rather than actively strengthening memories, sleep may instead serve to downscale synapses in order to allow further memory acquisition (Tononi and Cirelli, 2006). Moreover, Poe and collaborators demonstrated that this process occurs when cells fire at the trough of the theta cycle during REM (Poe et al., 2000), which leads to depotentiation of synapses (Pavlides et al., 1988; Huerta and Lisman, 1995). Interestingly, we observed that young SR animals displayed less relative theta power during REM, a deficit that might have limited the ability of these animals to update object/place representations.

Similarly to a previous study showing age-related impairments in OPR (Wimmer et al., 2012), we observed performance deficits in old control mice. However, we demonstrated that these performance deficits were different from those found in young SR mice. In old mice, “context” cells showed long-term instability, remapping between the training and test session, whereas young SD mice display stable representations of context. Reduced spatial stability has been reported in old animals (Barnes et al., 1997), along with impairments in several spatial tasks (Rosenzweig and Barnes, 2003). Therefore, it is possible that the sleep fragmentation observed in old control mice contributes to impair consolidation of the static aspects that conform an environment.

The observation that SR has a positive effect in old animals suggests that as long as animals are able to consolidate NREM during the first 10 hr after training, memories can be properly stored. These results suggest that the time window for memory consolidation may be different in young and old animals. It is well established that in young animals, the initial hours after training are critical for initiating transcriptional events that lead to the translation of new proteins important for memory encoding (Bailey et al., 2004). In agreement with this, we do observe more spindles, which are important for consolidation, during the initial hour post-training in young animals. However, converging lines of evidence indicate that the time window for

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protein-synthesis dependent memory consolidation is extended in old animals (Schimanski and Barnes, 2010). Moreover, while sleep restriction inhibits translational processes in young mice, it does not affect protein synthesis in old animals (Naidoo et al., 2008). Together, these observations suggest that NREM consolidation and increased spindle numbers during sleep recovery may have beneficial effects in old mice because these changes occur when the window for memory consolidation is still open. One interesting future line of inquiry would be to conduct sleep restriction 5 hr after training. We predict that this manipulation may not affect young animals but will significantly impact old mice in which prolonged time windows are necessary for consolidation.

Finally, spindles have been associated with memory encoding (Ulrich, 2016; Antony et al., 2019) and alterations in spindle count and properties are good predictors of age-related cognitive decline (Taillard et al., 2019). Our data support these findings since young controls display higher spindle counts of longer length than old controls during the first hour post-training. However, we also show that increases in spindle count during sleep recovery are sufficient to enhance OPR memory, demonstrating that manipulations that improve sleep architecture even 5 hr after learning can ameliorate memory deficits. In summary, our findings contribute to a better understanding of the effects of sleep quality on memory and hippocampal representations, and have potential clinical implications for rescuing age-related cognitive deficits.

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Figure Captions

Figure 1. Timeline and performance in the OPR task. A) Schematic of behavioral design. B) Percent change in preference for displaced object for all groups. C) Object exploration times during training and testing. Young control (n=16), young SR (n=15), old control (n=13), old SR (n=15). Histograms represent mean ± standard error of the mean (SEM).

Figure 2. Schematic of electrode placements and changes in firing rate during the OPR task. A. Schematic of electrode placements and microphotograph showing example of lesion marking electrode placement in CA1. B-D) Mean (B), peak (C), and out of field (D) firing rate for all groups across trials. There were no differences on average measures of firing rate across the groups throughout training. However, average firing rates during the initial object exposures (T1 and T2) were higher than average firing rates during habituation and test trials ($p<0.05$) for all groups. No differences were observed across training trials ($p>0.05$). E) Rate remapping - absolute differences in peak firing rate across trials - was calculated for all groups during training and testing. Results indicated that young controls and old SR mice displayed higher rate remapping in comparison to young SR and old control groups during the test trial. Histograms represent mean ± SEM.

Figure 3. Spatial information content, global remapping across sessions, and percentage of different cell types across groups during performance in the OPR task. A) The spatial information content of cells from old SR animals increased during testing in comparison to cells from old control mice ($p<0.05$). However, there were no significant differences between old SR, young control and young SR mice ($p>0.05$). B) Average global remapping for all groups across trials. Old animals displayed significant more instability than young mice between the habituation and trail 1 ($p<0.05$). During testing, old control mice displayed more instability that all the other groups ($p<0.05$). C-D) Percentage of context, object, and unstable cells recorded in young (C) and old (D) mice. Young controls (n=6 mice, 60 cells), young SR (n=4 mice, 50 cells), old control (n=5 mice, 42 cells), old SR (n=5 mice, 37 cells). Histograms represent mean ± SEM.

Figure 4. Cell-type remapping across sessions during performance in the OPR task. A) schematic indicating how trial correlations were computed. B-D) Average global remapping of context (B), object (C), and unstable (D) cells across trials (left) and corresponding examples of color coded place cell rate maps (right). Note that context cells are only unstable in the old control group, whereas object cells are unstable in all groups except the young SR animals. Waveforms shown recorded on day 1 and 2 show high similarity, indicating recording stability during the 24 hr period of training and testing. Number on top of each map represents peak firing frequency used to normalize the data. Context cells: Young controls (n=23 cells), young SR (n=20 cells), old control (n=9 cells), old SR (n=8 cells). Object cells: Young control (15 cells), young SR (13 cells), old control (15 cells), old SR (16 cells). Unstable cells: Young control (22 cells), young SR (17 cells), old control (18 cells), old SR (13 cells). Histograms represent mean ± SEM.

Figure 5. Sleep patterns during post-training and recovery sleep periods in young and old mice. A.) Photograph of the sleep restriction chamber. B) Examples of EEG/EMG activity during WAKE, NREM, and REM. C-E) Percentage of total time (C), number of bouts (D), and bout length (E) in Wake, NREM, and REM during post-training sleep in young and old control mice. F-H) Percent total time (F), number of bouts (G), and bout length (H) in Wake, NREM and REM during recovery sleep in control and SR groups. Young control (n=11), young SR (n=10), old control (n=8), old SR (n=10). Histograms represent mean ± SEM.

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Figure 6. Relative delta power (RDP) during NREM and relative theta power (RTP) during REM. A-B. RDP during post-training (A) and recovery sleep (B). Although there were no significant differences in RDP between young and old controls during the post-training sleep period (A), RDP increases in young SR mice during recovery sleep (B). Band range: low: 0.25-4 Hz. C-D. RTP during post-training (C) and recovery sleep (D). There were no differences between young and old control mice in RTP during the post-training period. However, RTP was significantly reduced in young SR mice in comparison to young controls ($p<0.05$). Histograms represent mean ± SEM.

Figure 7. Spindle characteristics. A-B. Average spindle count (A) and duration (B) during the post-training period. No differences were found between the groups. C-D. Spindle characteristics during 1 hr segments of post-training sleep. Young control mice displayed more spindles (C) of longer duration (D) than old controls during the first hour post-training. E-F. Average spindle count (E) and duration (F) during the recovery period. Old SR mice displayed more average number of spindles than old controls ($p>0.05$). F. No differences were observed in spindle duration. G-H. Spindle characteristics during 1 hr segments of recovery sleep. G. Old SD mice displayed more spindles than old controls across all segments ($p<0.05$). Additionally, all groups displayed more spindles during the first two hours of recovery sleep in comparison to the last 3 segments ($p<0.05$). H. No significant differences in spindle duration were observed across the groups during the 1 hr recovery segments. Histograms represent mean ± SEM.

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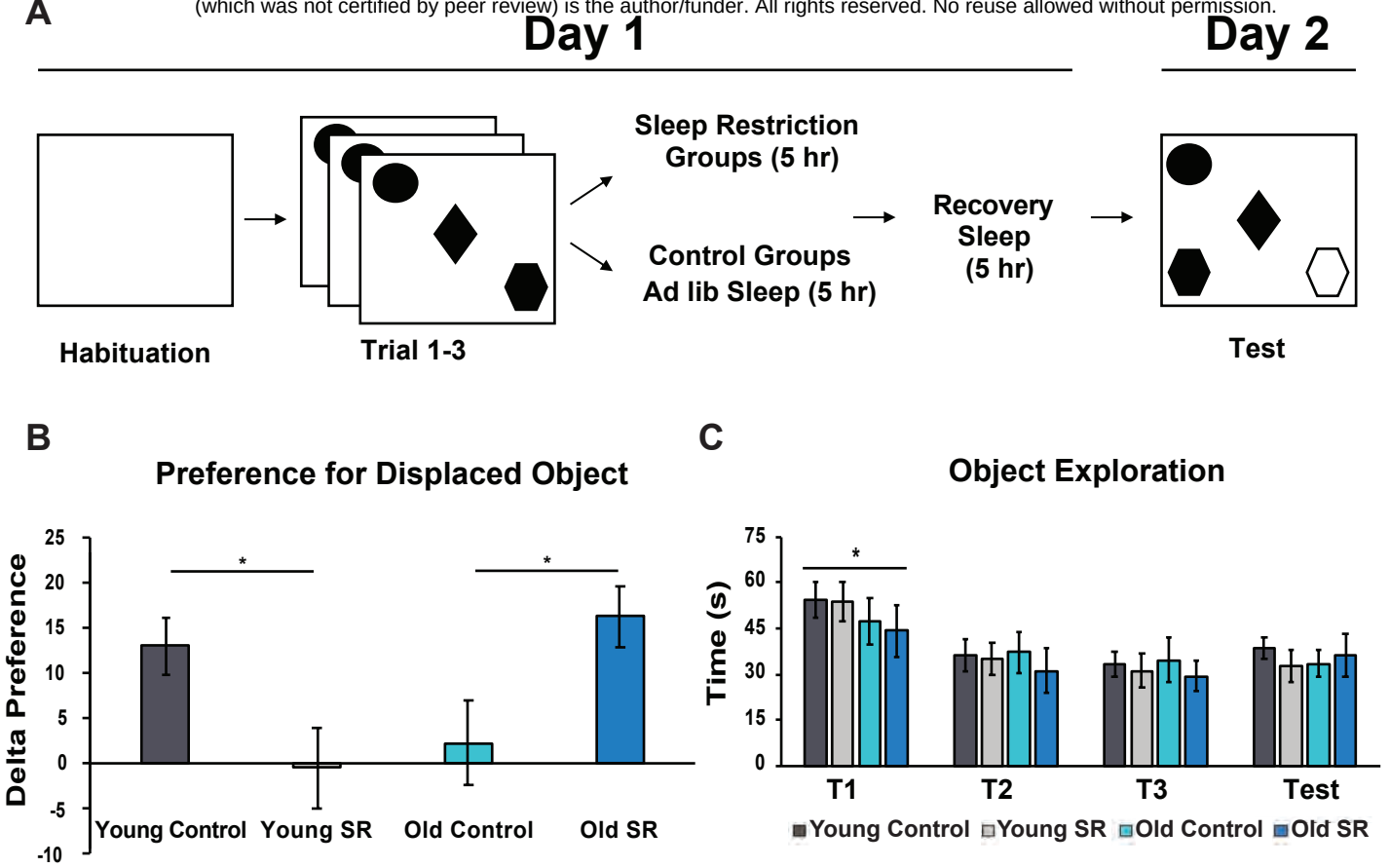

Figure 1

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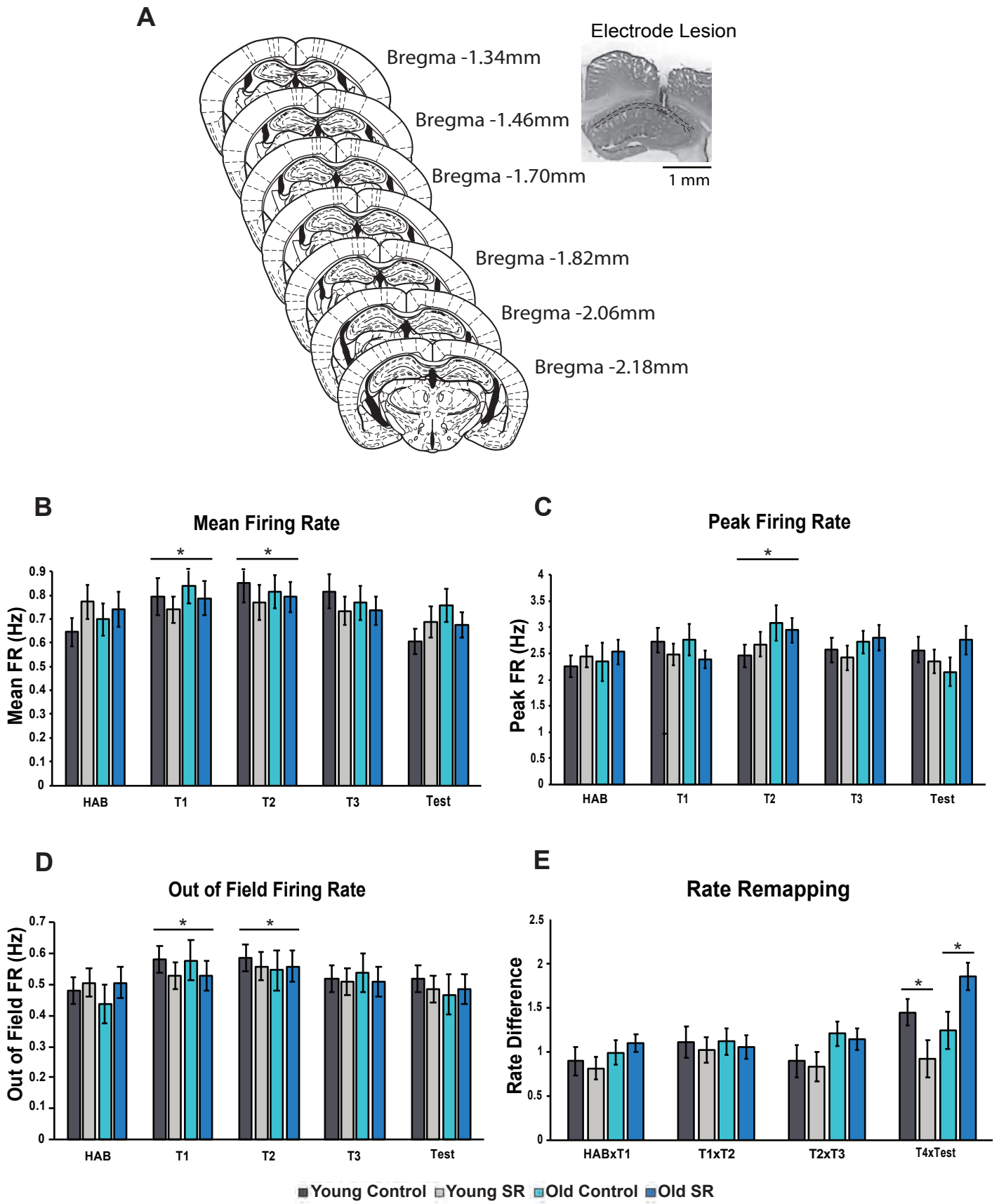

Figure 2

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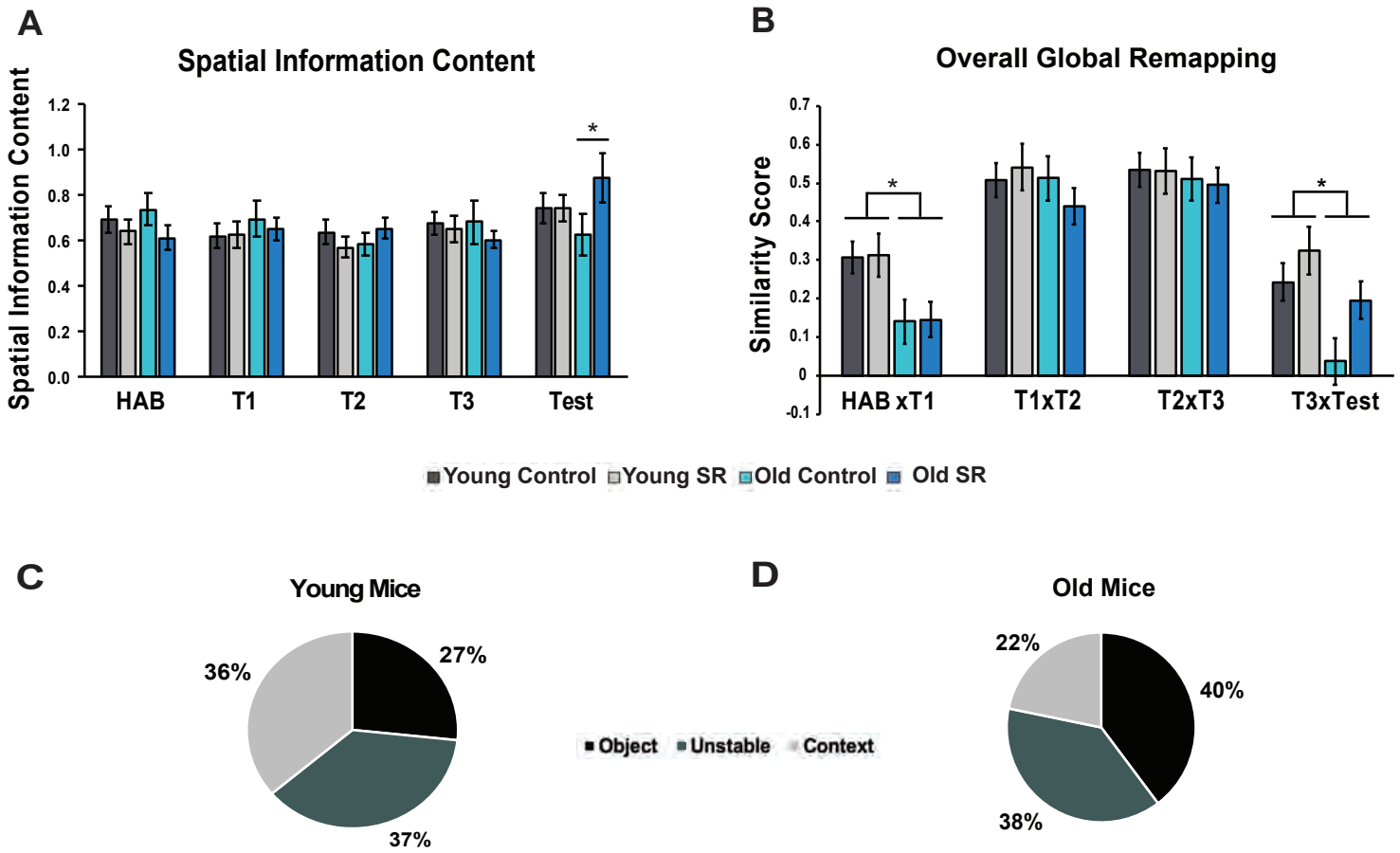

Figure 3

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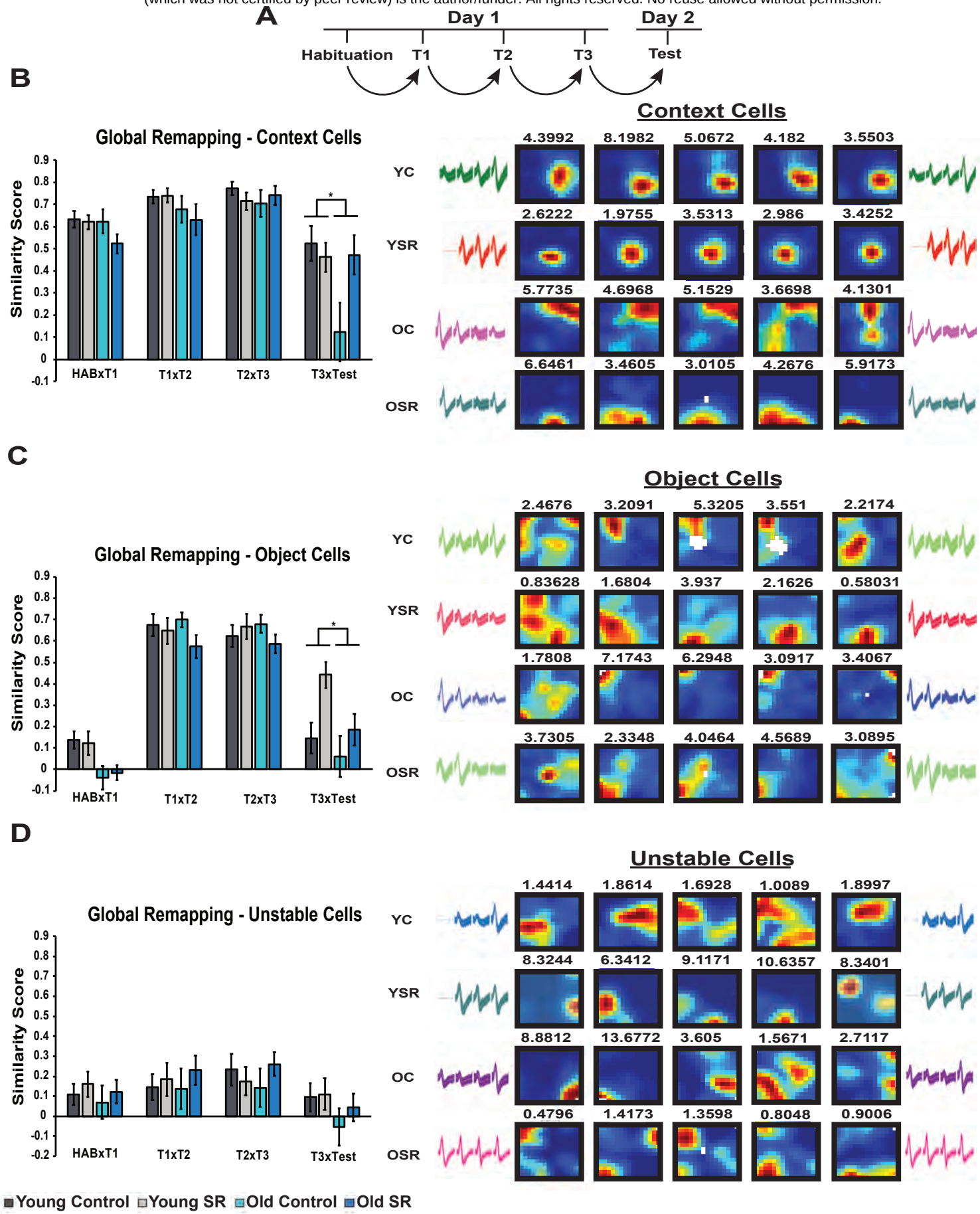

Figure 4

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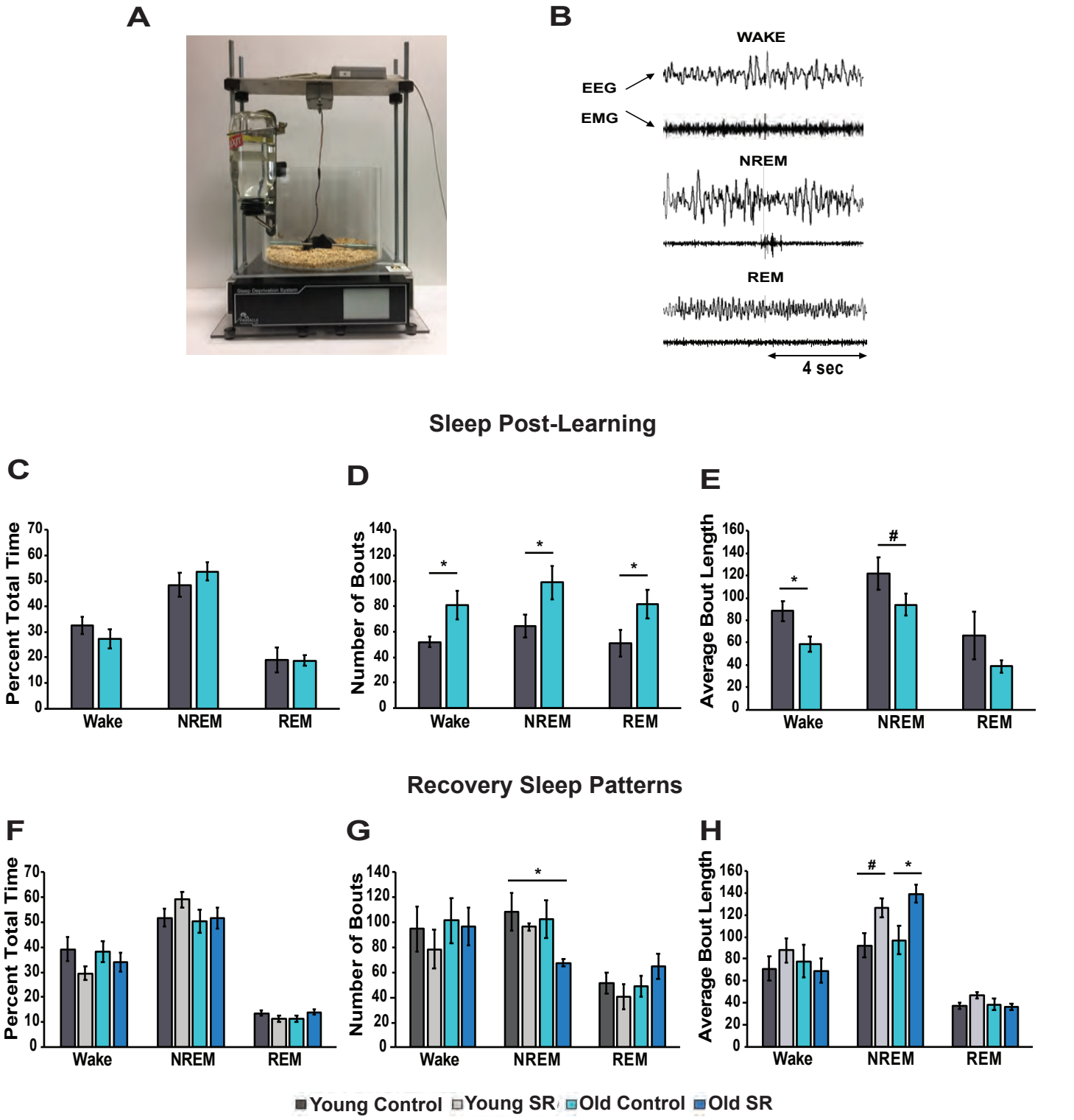

Figure 5

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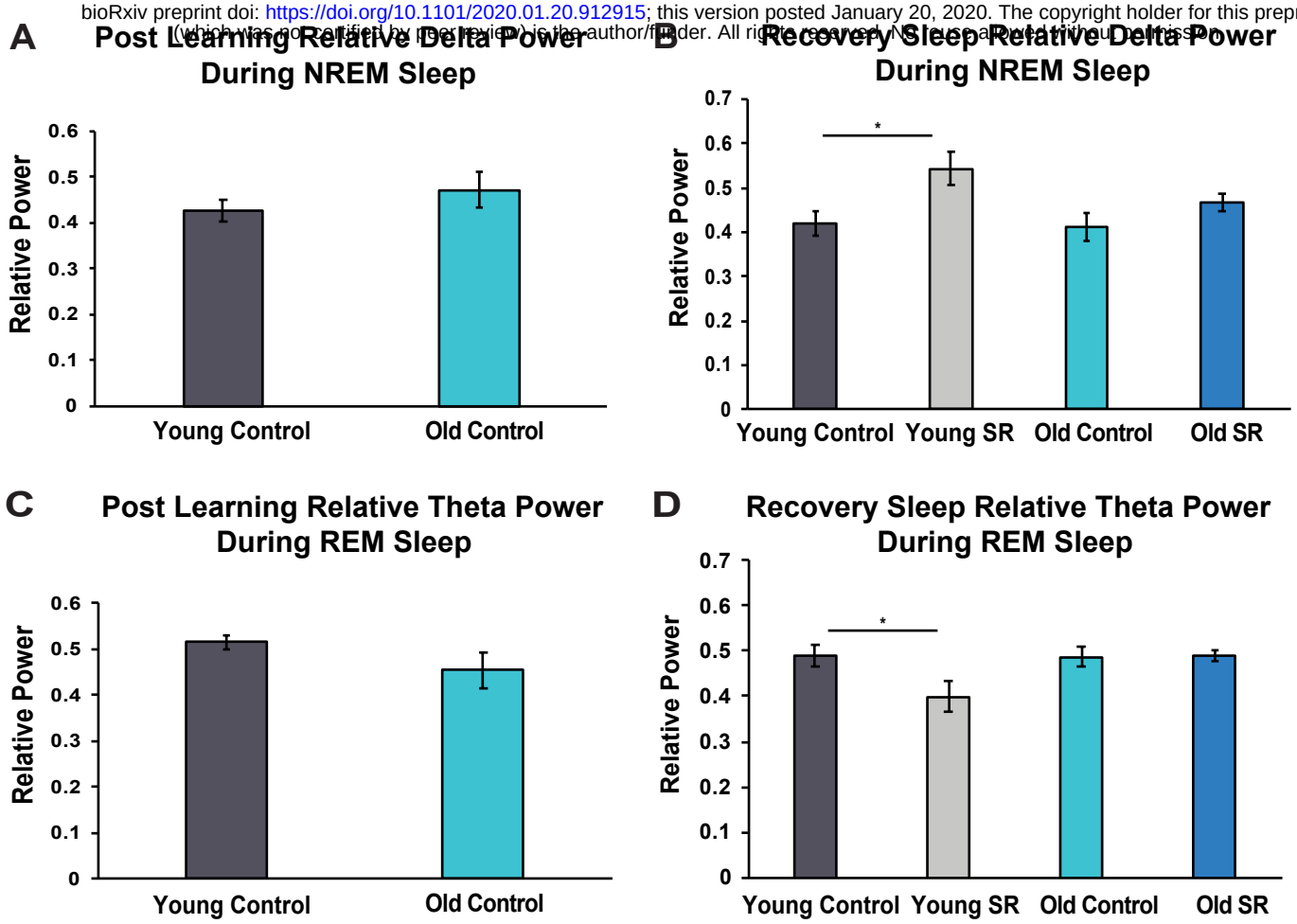

Figure 6

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Post-Learning Sleep

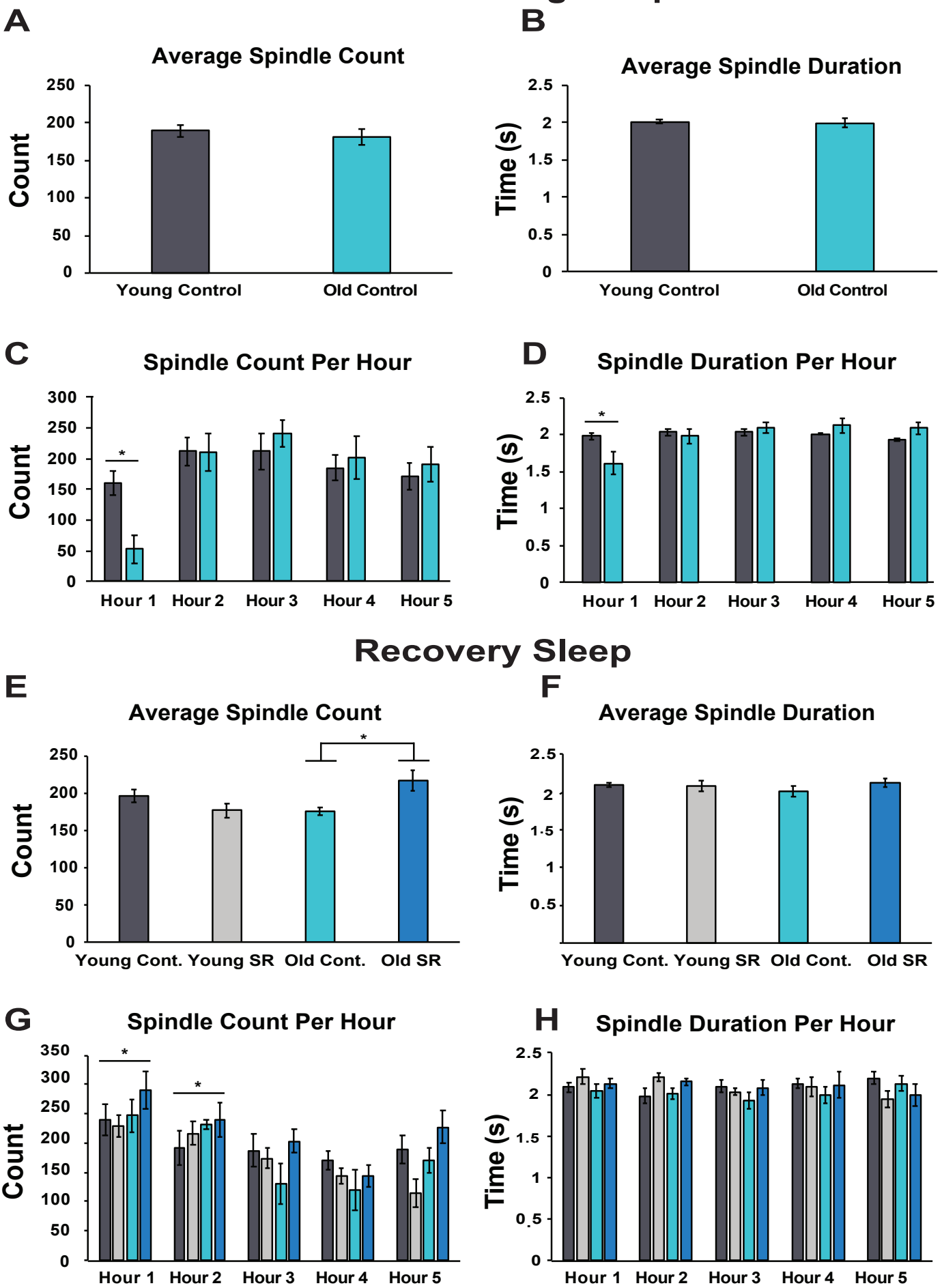

Figure 7