Prevalence and modulation of rat off-track head-scanning on linear tracks: possible implications for representational and dynamical properties of hippocampal place cells

Payton J. Davis[1,2 *], Stephen T. Jones[1 *], Francesco Savelli[1]

[1]Department of Neuroscience, Developmental and Regenerative Biology
University of Texas at San Antonio, San Antonio, TX, USA

[2]*Present Address:* Department of Neurobiology, Neuroscience Institute
University of Chicago, Chicago, IL, USA

[*] These authors contributed equally

Send correspondence to: Francesco Savelli, fsavelli.research@gmail.com

ABSTRACT

(Re)mapping of different environments by hippocampal place cells is thought to reflect incidental learning. Rat “head scanning” is a spontaneous and presumed investigatory behavior that can trigger the onset of firing locations in place cells. This behavior was studied on (quasi-)circular tracks, and it was speculated that off-track head scans might have been overlooked or inadvertently discouraged in studies employing more common apparatus. To better understand the general prevalence and significance of off-track scanning, we investigated it in rats running laps on linear tracks in rooms featuring visual landmarks. Rats scanned along the entire length of the track, even in highly familiar conditions and when they were rewarded only at the two ends of the track (albeit less than rats rewarded throughout the track), demonstrating that co-localized rewards are not necessary for the occurrence of this behavior. Scanning rate increased markedly in a novel room and then declined steeply during each daily session in this room over 3 days. Partial daily boosts transiently counteracted this decline, producing a “seesaw” profile that is reminiscent of previous observations on rat rearing or place cell plasticity. Moreover, the remapping that place cells are known to undergo in similar context changes could conceivably be facilitated by the putative surge of new place fields induced by increased scanning. Hence, investigatory behaviors could be causally involved in the representational and dynamic properties of hippocampal representations. Addressing these possibilities could help uncover the learning processes governing the incidental creation and update of a cognitive map.

KEYWORDS: head scanning, exploratory behavior, novelty, rearing, cognitive map, place cell

HIGHLIGHTS:

- Rat head scanning is known to trigger the onset of firing locations in place cells
- Off-track scanning occurs on linear tracks in familiar and novel conditions
- Co-localized rewards are not necessary for its occurrence
- Response to a novel room resembles that seen in rearing and place cell dynamics
- These relationships could help uncover the incidental process of map making

INTRODUCTION

Hippocampal place cells collectively establish independent maps across different environments, contexts, or behavioral conditions (Bostock et al., 1991; Moita et al., 2004; Leutgeb et al., 2005; Wills et al., 2005; Alme et al., 2014). This (re)mapping process is thought to reflect learning that "is *incremental* …, *latent* because it does not necessarily manifest in behavior, and *incidental* because it takes place in the absence of explicit reward" (O'Keefe, 2007, Ch 11, p. 514, emphasis in original). While examples of behavioral *consequences* of place cell firing remain rare (Lenck-Santini et al., 2002; Gagliardi et al., 2024), it is possible that *spontaneous exploratory and investigatory* behaviors support the presumed incidental creation or update of place cell maps (Lever et al., 2006). The exploration of a novel environment by rodents is characteristically segmented into alternating stops and bouts of forward progression (Golani et al., 1993; Drai et al., 2001; Eilam et al., 2003).

It has long been observed that head movements could reflect internal processing at experimentally defined decision points (Tolman, 1948; Redish, 2016; Kane et al., 2024). Crucially, "head scanning" is also the only documented case of a spontaneous and discrete behavior that can abruptly elicit the onset of new hippocampal place cell firing at arbitrary locations of the environment (Monaco et al., 2014). In the most intriguing display of this phenomenon, a cell fires when the rat samples an *off-track* position by stopping and peering over the track edges—presumably to inspect distal cues. The cell then begins firing consistently in the adjacent location *on the track* in every consecutive lap, thus exhibiting a persistent "place field." The authors noted how the one-trial character of this phenomenon might reflect the type of neural modifications expected of discrete spatial updates of the map or—more generally—episodic memory function.

Off-track scanning and its impact on place cells and cognition have been characterized only in two studies employing specific types of circular/hexagonal tracks and experimental protocols (Monaco et al., 2014; Rao et al., 2021), leaving questions open about its general prevalence and functional relevance. In particular, it was speculated that this behavior might have been overlooked on linear tracks (e.g., by clipping trajectories exceeding the track edges) or inhibited in rats traversing the track ballistically when food rewards are restricted to its two ends (Monaco et al., 2014). Still, much of what is known about place fields—from their early characterization (O'Keefe and Speakman, 1987) to their coding properties (McNaughton et al., 1983; O'Keefe and Recce, 1993; Muller et al., 1994), topography (Kjelstrup et al., 2008), statistical distribution (Rich et al., 2014), plasticity (Mehta et al., 2000; Frank et al., 2004), and spontaneous remapping (in mice; Zheng et al., 2024)—has been learned over multiple decades in numerous studies employing mazes comprising linear segments that were inspired by the rodent navigation literature (Wijnen et al., 2024). Therefore, it seems critical to know whether and how the functional insights gained from the examination of head scanning could retrospectively apply to the existing place cell literature and guide future studies, especially considering that modern approaches often employ VR strategies based on fixing the animal's head. Hence, we set out to investigate the expression of off-track head scanning in rats trained to either obtain food rewards at the two ends of a linear track (as common with this apparatus) or throughout the track (as was done in the previous head scanning studies) in both familiar and novel conditions.

METHODS

Subjects

Long-Evans rats were obtained from Inotiv Laboratories and allowed to acclimate in the UTSA Laboratory Animal Resource Center vivarium for at least seven days after arrival (Fig. 1A). Animals were kept on a 12hr dark/12hr light cycle, and all handling, training, and experiments were conducted during the light phase of the cycle (07:00 – 19:00). Female rats were ~eight weeks of age (175 – 199g), and male rats were ~five weeks of age (200 – 224g), at time of arrival to the animal facility. The difference of age was intended to reduce the weight gap between males and females. Animals were balanced with respect to sex (6M, 6F), though two male rats (Rat03 and Rat09) only completed a partial experimental protocol (Table 1). Animals were weighed daily. During the training and experimental phases, animals were placed on food restriction to motivate foraging behavior in the experimental apparatus; their weights were maintained at >80% of their ad libitum weight. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas at San Antonio**.**

Apparatus design

Animals were trained on a linear track (250cm x 12.5cm x 1.8cm, elevated 55.5cm from the ground) that followed one of two designs: a black-painted track supported by two grey metal legs that were placed perpendicular to the track at its two ends (n=4), or a grey-painted track supported by black-painted wooden horse-legs at its two ends (n=8). The track design was modified between the first (Rats 01-04) and following cohorts of animals (Rats 05-12) due to excessive dwelling at the ends of the T-arms of the first track design. Moreover, the first cohort was tested on the same track in all sessions, even when they occurred in different rooms (see below). For all other rats, identically built tracks were used in different rooms, and these tracks were not moved from room to room.

Our experimental design employed two rooms: Room A and Room B (Fig. 1B). Animals were randomly assigned to either room for training and were balanced between the two rooms. Room A was slightly larger than Room B (Room A: 3.05x3.76 m with a 0.43x0.30 m column in the top-right corner of the room (11.34 total m^2); Fig. 1B top row; Room B: 2.74x3.18 m (8.71 m^2); Fig. 1B bottom row). Room A cues consisted of black curtain panels covering wooden shelves along the walls, a 1.5m tall roll of brown butcher paper standing next to a computer desk, a small black camping chair, a standing lamp, a coat rack covered by a dark table cloth, a black cardboard rhombus wall cue, a rectangular cardboard wall cue with the outer perimeter painted black, and a triangular cardboard wall cue with large black dots (Fig. 1B top row). Room B cues also consisted of a computer desk, standing lamp, black curtain panels covering wooden shelves along the walls, and triangular cardboard wall cue that was instead painted fully black. Room B additionally had a black L-shaped wall cue and a black circular ring (Fig. 1B bottom row).

To encourage animal behavior, illumination was kept at levels comparable to previous recording studies that found spatial cells responsive to similar wall cues manipulations (Monaco et al., 2014; Savelli et al., 2017).

Behavioral Training Protocol

After the 7-day acclimation period, animals underwent pedestal and handling training for a minimum of 4 days, wherein they were placed on a circular plate attached to a wooden platform secured by a clamp to a cinderblock (Fig. 1A). The animals remained there for a minimum of 30 minutes, after which they were placed gently onto the laps of laboratory researchers and handled for a minimum of 5 minutes. During the initial handling period, animals were returned to their home cages with water and food *ad libitum*. Behavioral training consisted of at least four days of initial exposure to the linear track apparatus in one of the two rooms, Room A or Room B in their standard (STD) cue configuration (Fig. 1B, left column). Animals were counterbalanced by training room to ensure that the effects analyzed in this study could be attributed to the novelty manipulations rather than room-specific characteristics. 25mg chocolate-flavored food pellets from BioServe (Flemington, NJ) were placed randomly over the length of the track for the animals to consume. Animals were removed from their home cage, placed onto the pedestal, and transported into the experiment room. The pedestal was then placed on a cinderblock that remained fixed in the room, and the rat was removed from the pedestal and placed on the end of the track nearest to the door. For the initial exposure period, animals were allowed to explore the track with unrestricted food access (animals consumed an average of 0.5 – 2.5g per session) for at least 20 minutes daily. Animals were placed on food restriction after the first exposure session and remained on food restriction until the end of the study.

After the animals had completed four initial exposure days and could consistently traverse the length of the track several times over 20 minutes, they transitioned to the next training phase. During this phase, animals were tasked to run at least 30 laps in a single session. The food on the track was restricted to an average of approximately six pellets per lap, for a total of 3.5 – 4.5g per session. We discouraged doubling back by blocking the animals with a laboratory notebook when they began prematurely reversing direction. Excessive grooming and prolonged rests were discouraged by loudly snapping or clapping. All laps were counted using a digital smartwatch or clock that kept track of total elapsed time and lap duration. Once animals were able to complete a set of 30 laps in a single session with smooth and sustained progression (i.e., minimal or no grooming, prolonged rests, or premature attempts to reverse running direction) for a minimum of three training days, they were then tasked with running two identical sessions in a single training day that met all criteria that were described previously. Animals were returned to their home cages after each session with access to water for a minimum of 5 minutes before beginning the next session. After at least 1-2 days of successful double-training sessions, the animals had to complete three consecutive sessions of 30 laps in a single training day. Animals that completed three consecutive sessions of 30 laps across three consecutive training days were allowed to begin the testing phase, which lasted six days. Data from these last 3 training days constituted the PRE phase we used as baseline in our analyses (Fig. 1A, explained below, in 4/266 sessions included in analysis, the rat did not finish the 30th lap).

All rats from the first cohort (n=4) were trained simultaneously in an open field environment (100cm x 100cm x 50.5cm box, same food type) during a concurrent study (Table 1). They were trained for 45-60 minutes per day after track training. All animals were retired from box training before the PRE (REF) training phase of the present study.

Testing Protocol

The testing phase consisted of two main experiments: cue manipulation (CM) and room change (RC). Each experiment lasted three days, and all animals completed the CM experiment before beginning the RC experiment. Both experimental conditions followed the same general protocol as the training sessions described previously encompassing 3 daily sessions of 30 laps; however, the second standard (STD) training session was instead a CM or RC session (Fig. 1A). In a CM session, the rat was run in the same room where it was trained, after the distal cues were manipulated: two cues were swapped, one cue was removed, and at least one panel of curtains in the room was raised above the shelf it was attached to, therefore exposing wooden shelves that were previously not visually accessible to the rat (these cue-manipulations were room-specific and remained the same for each room for the entire duration of the experiment and for all rats; Fig 1B right column). After the CM session, all cues were returned to the familiar, STD configuration so that a third (last) daily session was run in this configuration. Data from this third session were not analyzed in this study. Animals completed three days of CM experiments before they began RC experiments. After the animals completed the first STD session in their training room, the second (RC) session was conducted in the opposite room under the STD conditions for that room. For example, an animal trained in Room A would complete a session in Room A with STD Room A cues and then be moved to Room B to complete the second session with STD Room B cues (Fig. 1B left column). Animals were not disoriented or otherwise perceptually screened during the relocation process to and from the novel room. All animals were retired from the study and removed from food restriction after completing three days of RC experiments. For both CM and RC test days, we associate a serial number to each session, reflecting its position in the sequence of sessions run on a given day, irrespective of the type of session. Thus, a typical experiment day will include a sequence of STD1, CM2, and STD3 for the cue manipulation condition or STD1, RC2, and STD3 for the room change condition, while during the PRE phase we had STD1, STD2, STD3.

Video Collection and Analysis

Video data was captured using an Allied Vision Manta 1.58-megapixel digital camera with an F/1.8 aperture, 1.8-33.0 mm focal length lens (Theia Technologies) mounted 2 m above the track. This data was then recorded using the Trodes video capture system (SpikeGadget, Inc.) and analyzed using DeepLabCut (Mathis et al., 2018). Videos were recorded at 7Hz (to ensure proper camera exposure) or downsampled at this frequency prior to DeepLabCut analysis to ensure a homogeneous treatment of the data.

To apply DeepLabCut-automated tracking to our video data, approximately 15 frames of 1 – 3 videos per animal, comprising 34 videos in Room A and 33 in Room B, were first manually annotated by labeling the ears, nose, and tail base of the animal when visible in each frame. Then 95% of these frames were used for training a resnet50-based neural network, with default parameters. Room A and Room B networks were refined through several rounds of correction-retraining phases to minimize artifacts. We extracted individual example frames that were labeled incorrectly by the model and manually corrected the labels before retraining the network. This process was repeated until automated tracking was deemed satisfactory. We used a p-cutoff of 0.6 as the acceptance threshold for each tracked feature (valid labeled position). This threshold was applied conjunctively to both ears, with the midpoint used to determine the head position of the animal. Videos from training, standard, and experimental sessions were then analyzed using the trained networks.

Identification and Characterization of Scanning Events

We implemented a custom algorithm to detect laps and off-track scans from position data. We began by parsing the position data obtained through DeepLabCut, filtering out any positions marked as lost or invalid. We then delineated specific areas of interest on the track, namely the outer edges and the two ends (measured 25 cm from either end). Laps were defined as trajectories beginning at one (the starting) end of the track, moving to the far end, and returning. We iterated through the trajectories to identify the beginning and end of laps, including both the outward (leaving the starting end) and inward (returning to the starting end) portions. Due to differences in apparatus and study design, our off-track-scan detection algorithm differs from the one originally developed by Monaco et al. (2014). Briefly, their algorithm first identified non-running pauses, defined as instances where forward movement was less than 10° clockwise per second, during which radial distance and velocity deviated from the preceding 4 seconds of lateral movement. These pauses were then assessed as scanning events if they exceeded a radial distance of 2.5 cm from the track edge and lasted longer than 400 ms from when the animal left the track until it returned. Consecutive events occurring within 200 ms of each other were merged into a single event.

Our simplified criteria focus on off-track excursions of the animal. We first defined putative scanning events as instances where the midpoint of the animal's ears exceeded the bounds of the track by at least 2.5 cm for a minimum of 500 ms. For each identified putative scan, we recorded the start and end timestamps and measured the magnitude, or maximum distance from the edge of the track. We categorized scans as occurring at either the starting or far end of the track, or on the left or right side of the middle of the track during the inward or outward-bound portion of a lap. These putative scans were then reviewed, with outliers manually flagged and removed based on inconsistencies in magnitude and velocity when compared to the animal's trajectory as observed in the video. This step was crucial to avoid inflating the total number of scanning events.

Because animals generally tended to scan disproportionately more frequently at the two ends of the track, we automatically excluded scanning events at these locations from most analyses presented in this study. Excluding scans at the track ends allowed for a more insightful comparison with previous studies conducted on continuous tracks (e.g., circular or hexagonal tracks) that do not have finite endpoints (Monaco et al., 2014; Rao et al., 2021). Thus, our analyses primarily focused on scanning occurring during progression along the track, except for those quantifying how scanning changes over time/distance traveled, for which we considered all events, including those occurring at the two ends of the track, to avoid temporal gaps.

Data Analysis

Data are reported as means and standard errors, and parametric statistical tests (paired and unpaired two-tailed t-tests) were used on all data unless otherwise stated. We used an alpha level of 0.05 for all statistical tests. Data analysis and plotting were implemented in Python using open-source libraries (Numpy, Scipy, Pandas, Matplotlib, ResearchPy) made available through the Anaconda distribution (Anaconda, Inc.).

RESULTS

We examined *off-track* sampling behavior in rats running laps on linear tracks for food rewards distributed either throughout the track ("all" rats; n=8) or at the two ends ("ends" rats, n=4), focusing primarily on scanning events occurring along the *main segment of the track*. We quantified this behavior in three conditions of varying environmental novelty in 12 rats: (1) the familiar room ("PRE" training days), (2) the same room after several landmarks were removed or shuffled ("CM", cue manipulation test days), and (3) the alternate (novel) room that differed in layout and featured novel cues ("RC", room change test days) (Fig. 1B).

Scanning in a familiar environment on a linear track

We first asked whether rats would scan off the linear track even in a highly familiar context. We thus assessed the baseline level of off-track scanning behavior in the training room, prior to any CM or RC manipulation. We focused on the last three training days immediately preceding the CM days (PRE days, Fig. 1A), in all training room and food placement conditions. Fig. 2A shows six examples of PRE trajectories. These plots include off-track scanning events occurring at the two ends of the track. Because most animals scanned disproportionately at the two ends of the track (56.08% of scanning events), and for a more direct comparison to the prior studies using continuous tracks lacking endpoints (Monaco et al., 2014; Rao et al., 2021), we excluded scanning events occurring at the two ends of the track from the data analyzed and illustrated in this paper, except for the last section addressing the evolution of scanning rate. Two rats (Rat03 and Rat09) exhibited inconsistent progress in behavioral performance during training and experiment days and did not complete the experimental protocol, and thus were excluded from the analyses (see also Table 1).

Rats consistently scanned in the highly familiar training environments during PRE days (mean+-sem: 14.12+-1.33 scans per session; Fig. 2B). Even the animals rewarded exclusively at the ends of the track performed scans throughout the main section of the track (Fig. 2B). However, in line with considerations put forth by (Monaco et al., 2014), these rats scanned significantly less than rats rewarded throughout the length of the track (mean+-sem: ends, 6.1481+-0.7524 scans; all, 17.5397+-1.6877 scans; $t(88)=-4.3278$, $p=3.9684\times10^{-5}$). Female rats scanned more than male rats in our cohorts (mean+-sem: F, 15.16875+-1.0667 scans; M, 11.0556+-0.8091 scans; $t(266) = 2.8191$, $p = 0.0052$; but see methods for differences in age/weight.) Similar sex differences were reported in multiple species for rearing on hindlegs (as discussed in Lever et al., 2006), but another study reported less rearing in females (Sturman et al., 2018).

Scanning after Cue Manipulation and Room Change

Next, we assessed how the two novelty conditions (CM and RC) presented during the testing phase of the study influenced scanning behavior. We first compared scanning behavior during the first STD session of each CM testing day (STD1) and the immediately following CM session (CM2; the number associated with a session reflects its serial position order in the sequence of sessions performed in the day, e.g., CM2 was the second session run, even if it was the first time the rat was exposed to cue manipulation on that day; Fig. 1A). There was a small decrease in scanning in STD1 vs. CM2 (mean+-sem: STD1, 13.6897+-1.9483 scans; CM2, 10.1379+-1.3212 scans; paired samples t-test for all animals and CM days, $t(28)=2.7292$, $p=0.0109$). We reasoned that animals may tend to decrease their scanning over time or distance traveled. If so, they would simply scan less in each subsequent session, and this tendency might counteract any propensity to scan more in the manipulated condition. To take into account this possibility,

we first examined how scanning behavior changed between the first and second sessions of the PRE (non-testing) days. Animals indeed scanned less in the second PRE session (STD2; mean+-sem: 11.9333+-1.9587 scans) compared to the first PRE session (STD1; mean+ sem: 15.6+1.9200 scans; paired samples t-test for all animals and PRE days, t(29)=2.4252, p=0.02176). Given that this result suggests that animals may scan less during their second session of the day regardless of session type, we attempted to unmask how the novelty of the CM condition could still promote scanning by comparing the changes in scan counts from the 1st to the 2nd daily sessions in PRE vs. CM days (Fig. 3A). The changes in scan counts did not significantly differ (mean+-sem of paired differences STD2-STD1 for all animals and PRE days: -3.6667+-1.5119 scans; mean+-sem of paired differences CM2-STD1 for all animals and CM days: -3.5517+-1.3014 scans; t(57)=-0.0575; p=0.9544). Thus, our CM condition failed to promote increased scanning behavior.

By contrast, the RC condition elicited a remarkable increase in off-track scanning in all rats (Fig. 3B). On average, there was a twofold increase in scanning between STD1 and the immediately following RC2 session (mean+-sem; STD1, 12.6552+-1.7266; RC2, 25.3793+-2.6455; paired samples t-test for all animals and RC days; t(28)=-4.9656, p=3.0471x10^{-5}). To better appreciate this phenomenon and take into account the tendency to decrease scanning between the first and second sessions observed in PRE and CM, we again compared changes in scan counts from the 1st to the 2nd daily sessions across PRE, CM, and RC conditions (Fig. 3A). These changes were greater in RC days (mean+-sem of paired differences RC2-STD1 for all animals and RC days: 12.7241+-2.5625 scans) than in PRE days (mean+-sem of STD2-STD1 reported above; t(57)=-5.5540, p=7.6376x10^{-7}) or CM days (mean +- sem of CM2-STD1 reported above; t(56)=-5.6632, p=5.3323x10^{-7}). Thus, these paired changes are also considerably and significantly greater in RC, further confirming that our RC condition succeeded in promoting increased scanning.

To draw a more direct comparison between STD2, CM2, and RC2 sessions (i.e., just the 2nd daily sessions across conditions), we computed their mean total scan count over the 3 days of each condition respectively for all rats (mean+-sem: STD2, 11.93333+-2.5150; CM2, 10.4833+-2.1402; RC2, 24.8167+-4.1335; Fig. 3C top). Given the considerable variability in individual propensity to scan across rats (see STD2 means in Fig. 3C top), we also normalized the CM2 and RC2 means with respect to each rat's STD2 mean (normalized means+-sem: STD2, 1.0000+-0.0000 scans; CM2, 0.9129+-0.1218 scans; RC2, 2.4097+-0.4422 scans; Fig. 3C bottom). Differences between STD2 and CM2 scan counts were not significant (absolute values, t(9)=0.9164, p=0.3834; normalized by STD2 means, t(9)=0.7153, p=0.4926). However, when STD2 and RC2 absolute scan counts are compared for each animal, all but one animal increased their scanning behavior during the RC sessions (absolute values, t(9)=-3.0211, p=0.0145; normalized by STD2 means, t(9)= -3.1878, p=0.0110; Fig. 3C). Additionally, direct comparison between RC2 and CM2 shows that all animals increased their scanning and yields analogous significant differences in scan counts (absolute scans, t(9)=-3.9340, p=0.0034; normalized scans, t(9)=-3.6352, p=0.0054; Fig. 3C). Note that the animals were counterbalanced by training room, to ensure that the impact of the RC condition we observed was due to novelty rather than room-specific features; there was no difference in scanning between the two rat groups tested in different rooms (absolute means+-sem: Room A RC2, 24.1+-8.4905 scans; Room B RC2, 25.5333+-2.1307 scans; t(8)=-0.6876, p=0.5111). Also note that the increase of scanning in RC quantified above was largely consistent in our complete set

of animals varying by apparatus, room, reward location, sex, and individual tendency to engage in scanning behavior (Table 1, Fig. 3B,C).

Duration and magnitude of head scans

Beyond scan counts, we examined differences in the duration and magnitude of scanning events across STD2, CM2, and RC2. The duration of RC2 scanning events were not significantly different from STD2 scanning events, whereas CM2 scans were slightly but significantly shorter than STD2 scans (mean+-sem: STD2, 3.2202=-0.1622 seconds; CM2, 2.7846=-0.1260 seconds; RC2, 3.5072=-0.1358 seconds; STD2 vs. CM2, t(650)=2.0520, p=0.04057; STD2 vs. RC2, t(1092)=-1.2742, p=0.2029; Fig. 3D top). CM2 scan durations were significantly shorter than RC2 scan durations (t(1028)=-3.1524, p=0.0017).

We quantified the magnitudes of scanning events as the distance reached by the rat from the track edge (see methods). RC2 scan events had slightly but significantly greater magnitudes than CM2 scan events (mean +- sem: CM2, 5.0954+-0.1081 centimeters; RC2, 5.4979+-0.0639 centimeters; CM2 vs. RC2, t(1108)=-3.2982, p=0.0010; Fig. 3D bottom). STD2 scan magnitudes did not significantly differ from either CM2 or RC2 (mean+-sem: STD2, 5.3332+-0.0998 centimeters; STD2 vs. CM2, t(650)=1.6130, p=0.1072; STD2 vs. RC2, t(1028)=-1.4317, p=0.1525).

Lateral biases

We observed that animals tended to have a directionality bias in their scanning behavior, with more scans occurring on one side of the track than the other (Fig. 4A, additional examples of this phenomenon are in Figs. 2A and 3B). To verify and quantify this observation, we computed a laterality index (LI) for each session by computing the difference between the counts of rightward and leftward scanning events and dividing it by their sum. The left and right directions of the experimental room were allocentrically defined relative to an observer standing at the track's end closer to the doorway and facing the opposite room wall. LI values can range from -1 to 1, where negative values indicate a leftward bias, and positive values indicate a rightward bias.

During STD2 in PRE days, animals trained in Room B had a significant right-ward bias (LI, mean+-sem: 0.6744+-0.0724; t-test against zero: t(14)=9.3176, p=2.2257x10^{-7}; Fig. 4B top), whereas Room A animals did not have a clear preference (LI, mean+-sem: -0.0770+-0.1860; t-test against zero: t(14)=0.4142, p=0.6850; Fig. 4B top). The LIs in these two groups were significantly different when directly compared (t(28)=-3.7650, p=0.0008). During CM2 sessions, animals trained and tested in Room A had a strong leftward bias (LI, mean+-sem: -0.5207+-0.0986; t-test against zero, t(13)=-5.2813, p=1.4859x10^{-4}; Fig. 4B middle) as compared to animals trained and tested in Room B, which still exhibited a significant rightward bias (LI, mean+-sem: 0.8375+-0.0633; t-test against zero, t(14)=13.2292, p=2.6495x10^{-9}; Fig. 4B middle). LIs in these two groups was significantly different when directly compared (t(27)=-11.7540, p=3.9806x10^{-12}). During RC sessions, animals tested in Room B (trained in Room A) again had a significant rightward bias (LI, mean +- sem: RC2 Room B, 0.8036+-0.0584; t-test against zero, t(13)=13.7721, p=3.9405x10^{-9}; Fig. 4B bottom). Animals tested in Room A (trained in Room B) displayed a weaker tendency to scan towards the right side of the room (LI, mean+-sem: RC2 Room A, 0.2028+-0.1046; t-test against zero, t(14)=1.9387, p=0.0730; Fig. 4B bottom). Direct comparison between these two groups also yielded significant differences: animals tested in Room B (trained in Room A) had a significantly stronger rightward bias than

animals tested in Room A (trained in Room B) (LI, mean+-sem: Room A, 0.2028+-0.1046; Room B, 0.8036+-0.0584; t(27)=-4.9186, p=3.7899x10^{-5}; Fig. 4B bottom). Due to the track and cue location in each room (Fig. 1B), these biases can be roughly interpreted as being oriented toward the track side with more room clearance. This was also where the experimenter tended to dwell more often. So, the scanning lateral biases could be tentatively attributed to greater attention of the rat toward a better view of the distal landmarks or the experimenter.

Evolution of scanning rate over time and distance

Given the rats' tendency to scan less in consecutive sessions (quantified above), we were interested in the temporal evolution of their propensity to scan within and across days. Thus, we quantified changes in the rate of off-track head scanning over both time and distance traveled. To avoid temporal gaps, we did not exclude scanning events that occurred at the ends of the track in the following analyses and figures.

We first examined scan counts in one-minute time bins of (Fig. 5A). Given the high variability in scan counts between individual rats (Fig. 3C), the data in Fig. 5A is normalized relative to the mean scan count of the first minute of STD2 Day 1. Because session durations, too, vary across rats (Fig. 5B), in this approach the number of rats included in the time-bin averages starts decreasing beyond the duration of the fastest rat session, with the faster rats dropping out first, potentially skewing the interpretation of the scanning rate over time. Therefore, Fig. 5A is limited to the duration of the shortest sessions, focusing on the first six minutes of each session.

To overcome these limitations, we assessed how off-track scanning evolved over the distance traveled in the apparatus, as an alternative measure of exposure to environmental novelty on test days. We binned the number of scans occurring over increments of three laps. Again, given the high variability in scan counts across rats (Fig. 3C), we normalized these bins counts by the scan count for the first bin (laps 1-3) of STD2 Day 1 for each individual rat, before aggregating the data from all rats, so that each animal has equal weight in the curve (Fig. 5C). Across nearly all sessions, there was a significant decrease in head scanning from the beginning to the end of the session (mean+-sem: Laps 1-3, 1.6169+-0.1261; Laps 27-30, 0.6752+-0.0813; t(87)=7.9733, p=5.6076x 10^{-12}; repeated in individual sessions p<0.039, except STD2 Day 3 p=0.1533 and CM2 Day 3 p=0.3401). A general decline in scanning also took place over days in both CM and RC. Normalized mean scan count significantly decreased from the beginning of the first to the beginning of the third days of both CM (Laps 1-3, mean+-sem: CM Day 1, 1.6133+-0.2277; CM Day 3, 0.7467+-0.1676; t(9)=5.8720, p=0.0002) and RC testing days (Laps 1-3, mean+-sem: RC Day 1, 3.2204+-0.5507; RC Day 3, 2.1092+-0.3565; t(8)=2.9951, p=0.0172). The beginning of the first to third days of STD2 sessions did not significantly differ (Laps 1-3, mean+-sem; STD2 Day 1, 1.0000+-0.0000; STD2 Day 3, 1.0283+-0.2012; t(9)=-0.1408, p=0.8911). Intriguingly, the decline in scanning rate over days was partially counteracted by significant increases from the end of the previous day's session to the beginning of the next (mean+-sem: Day 1/2 Laps 27-30, 0.6228+-0.0967; Day 2/3 Laps 1-3, 1.4684+-0.1430; t(56)=-7.046, p=2.9064x10^{-9} repeated in individual transitions broken down by condition and day, p<0.017 for all comparisons except STD2 Day 1 vs. Day 2, p=0.0527). Hence, the overall evolution of scanning rate in RC over the 3 testing days took a "seesaw" shape (Fig. 5C).

DISCUSSION

Studies of navigation have long appreciated the circular problem of coping with spatial uncertainty in uncharted territory (Gallistel, 1990; McNaughton et al., 1995, 1996; Etienne et al., 1996; Kuipers, 2000; Thrun et al., 2005). Charting landmarks on a real or cognitive map requires positional estimates of the vantage points from which the landmarks are perceived, hence requiring self-localization; however, self-localization itself must be defined relative to, and rely on, the map under construction. The recursive, probabilistic solutions to this circular problem devised in robotics (Kuipers et al., 2004; Thrun et al., 2005) may be relevant to how the brain makes cognitive maps (Savelli and Knierim, 2019; Fischler-Ruiz et al., 2021; Thurley, 2021). These solutions *require active sensing oriented toward salient environmental features* integrated with path integration of self-motion cues (McNaughton et al., 2006; Jayakumar et al., 2019). Head scanning seems a behavioral strategy of broad ethological relevance well suited for this task (Jun et al., 2016). Our observations demonstrate the varying prevalence of this behavior on common apparatus in novel and familiar conditions and with different food-reward patterns. Moreover, they corroborate the notion that off-track head scanning can indeed reflect attention to environmental cues. Last, they highlight how the effects of this behavior on place cells (Monaco et al., 2014) offer an opportunity to uncover the putatively incidental and incremental learning processes governing how the brain constructs and updates cognitive maps.

Environmental influences

We observed a striking increase of head scanning frequency in rats exposed to a novel room ("RC" protocol), suggesting greater attention to the unfamiliar cues and different room layout. Moreover, our use of linear tracks uncovered lateral preferences in head scanning by animals exposed to the same room and condition. Head scans were generally biased toward the side of the room with more clearance. Distal/peripheral cues are known to have a stronger influence in setting the orientation of neural correlates of position and head direction in the limbic system (Cressant et al., 1997; Yoganarasimha et al., 2006). Thus, the lateral biases may have been modulated by the perceptual salience of distal cues. The experimenter could also have contributed to orienting the scanning behavior by dwelling more on the same side while dispensing food, though this factor cannot explain the overall increase of head scans in the novel room. Hippocampal neurons are known to encode aspects of the animal-experimenter interactions (O'Keefe and Dostrovsky, 1971; Snyder et al., 2024). Notably, our experiments were representative of how most place cell studies on linear tracks have been traditionally run, and we actively attempted to minimize these interactions during food placement. While off-track head scanning is prevented in most modern VR apparatus, approaches that avoid head-fixing (Hölscher et al., 2005; Lever et al., 2006; Aronov and Tank, 2014; Grosso et al., 2017; Madhav et al., 2021) could in principle be adapted to study what type of information is sought by the animal during scanning and its effect on place cells (Zeng et al., 2024), for example via closed-loop control of visual cues (Jayakumar et al., 2019; Madhav et al., 2024).

In "double-rotation" experiments head scan amplitude increased as a function of the mismatch angle between proximal (tactile) cues and distal (visual) landmarks (Monaco et al., 2014). While this phenomenon strongly suggests attention to landmarks as a driving factor in scanning, our partial cue manipulation in the highly familiar room ("CM" protocol) failed to increase the amplitude (or frequency) of head scans. Besides the difference in track geometry, our CM manipulation differed from the double rotation in several key ways that could have contributed to

this difference: (i) we did not disorient the rat between sessions; (ii) our cue mismatch only affected distal, visual cues, as opposed to the multimodal combination used in the double rotation; and (iii) we only manipulated a subset of the cues. In contrast, the double rotation was meant to induce an all-encompassing experience (capable of confusing even the human experimenter, personal observation by F.S.), in which every controllable cue belonged to one of two sets pitted against each other and with no background reference cue intentionally left uncontrolled.

Relationship with rearing on hindlegs

Parallels between scanning and rearing are worth considering in relation to landmarks. In rearing, the rat rises and stands on its hindlimbs, at times supported by a nearby wall, presumably to gain a better viewpoint or access to distal olfactory cues (Lever et al., 2006). Rearing appears involved in the recognition of distal landmark alterations by pre-weanling rats (Shan et al., 2023). Hippocampus inactivation is most effective at disrupting performance in a spatial task when performed concurrently with rearing episodes (Layfield et al, 2023). Manipulation of cholinergic inputs from the medial septum to hippocampus can alter rearing frequency or duration, possibly reflecting altered attentional states (Monmaur et al., 1997; Cassity et al., 2024). Both scanning and rearing can occur during theta rhythms in hippocampal Local Field Potentials (LFP). LFP theta is more prominent during scanning than non-scanning pauses, but less so than during locomotion (Monaco et al., 2014). The relationship between rearing and theta, however, seems more complex (Barth et al., 2018).

These considerations make it tempting to speculate that both scanning and rearing could be behavioral manifestations of the same internal drive toward seeking environmental information. Their concurrent investigation is somewhat complicated by the fact that rearing on narrow tracks lacking walls is more precarious and therefore occurs less frequently than in open arenas. Vice versa, rats routinely engage in rearing in open arenas, but extra-apparatus scanning would only be possible along the perimeters of an elevated platform devoid of walls. Both behaviors are consistent with the intermitted locomotion exhibited by rats exposed to a novel environment (as are on-track scanning events considered by Monaco et al., 2014 that we did not examine). Future studies could address the possibility of a common neuromodulatory origin (Concetti et al., 2024) and functional overlap between extra/intra apparatus head scanning and rearing. Besides affording better opportunities to acquire distal spatial information, any active pause in locomotion could also afford an effective window of integration and plasticity for generating a postsynaptic place field from entorhinal or intrahippocampal inputs (Savelli and Knierim, 2010; Bittner et al., 2015; Davoudi and Foster, 2019; Savelli, 2024). The activity of hippocampal spatial cells correlates with the position of animal's head (possibly the nose Huxter et al., 2008), and grid cells can anchor their firing patterns to remote visual cues lying beyond the boundaries of the navigable space (Gupta et al., 2014; Savelli et al., 2017). It is therefore conceivable that grid firing patterns could be extrapolated beyond these boundaries during off-track scanning, occasionally aiding in the potentiation of spatially adjacent inputs that would otherwise have remained too weak to generate a place field on the track (Savelli and Knierim, 2010). Indeed, scanning was observed to potentiate place fields in locations where feeble firing was often already present (Monaco et al., 2014), thus unmasking "dormant" place fields, which possibly reflect pre-existing spatial biases inherited from their inputs (McKenzie et al., 2021; Jones and Savelli, 2023; Savelli, 2024; Zheng et al., 2024). Beside grid cells, this process would likely include entorhinal correlates of environmental features possibly attended by the animal while

scanning (Savelli et al., 2008; Solstad et al., 2008; Deshmukh and Knierim, 2011; Høydal et al., 2019; Kinkhabwala et al., 2020).

Possible implications for place cell properties

The influence of head scanning on the emergence of place fields might extend to their representational properties. For instance, place cells display "global remapping" following context/environment changes similar to our RC condition (Leutgeb et al., 2005; Wills et al., 2005; Alme et al., 2014). Conceivably, a surge of new place fields induced by the marked increase in off-track scanning that we observed in this condition should contribute to remapping to some measurable degree. In environments preventing the animal from peering over the apparatus edges (e.g., in walled boxes), an equivalent influence could be exerted by intra-apparatus scanning (Monaco et al., 2014) or perhaps even rearing (Lever et al., 2006). [Rearing has been shown to modulate place cell firing (Barth et al., 2018), but whether it can induce persistent changes in place fields similar to scanning remains under-investigated.] As a possible illustration of this principle, place cell remapping was observed even in a familiar, unaltered environment, *after* the rat was subjected to auditory overstimulation in a separate sound chamber (Goble et al., 2009). This manipulation also resulted in extensive, scan-like deviations from the streamlined trajectories observed instead in control and baseline conditions (as suggested by the examples of Fig. 2 vs. Fig. 1 of that study). While this behavior may reflect a delayed anxiety/stress response rather than revived attention to the familiar landmarks per se, it could nonetheless have facilitated the observed place field remapping (e.g., by plasticity acting on new coactivation patterns of position-modulated presynaptic inputs as hypothesized above, which would not strictly rely on attention to landmarks). In addition to remapping induced by contextual or behavioral changes, place cells are also known to generate distinct maps for either direction of a track, a puzzling phenomenon that seems representationally analogous to global remapping across contexts but could result from large firing rate changes within place fields (Navratilova et al., 2012). As scanning can potentiate barely discernible firing locations into well-defined place fields (Monaco et al., 2014), it could contribute to some of these changes. (However, whether scanning-induced potentiation of place fields can proceed independently in the two running directions remains to be investigated, as it has so far been studied only in rats running around a circular track in the same direction.) Together, these considerations suggest a possibly underappreciated role of spontaneous investigatory behaviors in making/differentiating place-cell maps (though maintenance and context-based re-instantiation of each map must rely on different, mnemonic processes; Colgin et al., 2008). Exploring these possibilities could clarify the nature of learning thought responsible for (re)mapping: namely its incremental, incidental, and latent qualities discussed in the Introduction, especially in light of our demonstration of head scanning's relative independence from proximity to food rewards. An interplay of investigatory behaviors with other internal mechanisms postulated for remapping, such as concurrent changes in grid cell reference frames (Fyhn et al., 2007; Monaco et al., 2011) or autoassociative/attractor neuronal networks (Wills et al., 2005; Colgin et al., 2010), is also possible.

Similarly, the influence of head scanning on the emergence of place fields might also extend to their dynamical properties. We observed a decrease in scanning rate as the animal became more familiar with a novel room over 3 days. This decrease was transiently and partially counteracted by boosts in scanning at the beginning of the first test session of the day, and scanning rate would then decline steeply as the session proceeded. The resulting "seesaw" shaped decay over days is intriguingly reminiscent of that of the plasticity of place fields in novel

path configurations (Frank et al., 2004), of the emergence of direction sensitivity in place fields (Navratilova et al., 2012; discussed above), and of backward shift of place fields on tracks (Mehta and McNaughton, 1997; Mehta et al., 2000; Lee et al., 2004; which, however, would be more difficult to associate mechanistically to head scanning). Once more, it is tempting to draw a parallel with rearing, since preliminary observations suggest that its rate could follow a similar profile in novelty conditions (Lever et al., 2006). Fluctuations in the expression of these investigatory behaviors may thus be causally involved in dynamical aspects of place cell representations. Irrespective of a possible causal link, behavioral and neuronal plasticity still seem bound through shared dynamics induced by novelty.

AUTHORS CONTRIBUTIONS

Payton J. Davis: Designed experiments and analyses; Conducted experiments; Analyzed and interpreted data; Wrote manuscript. **Stephen T. Jones:** Customized behavioral data acquisition and animal trajectory extraction pipeline; Devised/tuned algorithms for lap and scanning detection, feature extraction, and visualization; Assisted with experiments; Gave feedback on analyses and manuscript. **Francesco Savelli:** Conceived study; Designed experiments and analyses; Wrote manuscript; Supervised all aspects of the study.

CRediT Format. **Payton J. Davis:** Writing—original draft, Writing—review and editing, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data Curation. **Stephen T. Jones:** Writing—review and editing, Visualization, Validation, Software, Methodology, Investigation, Data Curation. **Francesco Savelli:** Writing—original draft, Writing—review and editing, Supervision, Methodology, Formal analysis, Conceptualization.

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Rat ID	Sex	Training Room	Reward Location	Track Design	Track Training Duration in Days (excluding PRE)
Rat01	M	Room A	Throughout	T-arms	15
Rat02	F	Room A	Throughout	T-arms	15
Rat03*	M	Room A	Only at ends	T-arms	16
Rat04	F	Room A	Only at ends	T-arms	13
Rat05	M	Room B	Throughout	Horse-legs	12
Rat06	F	Room B	Throughout	Horse-legs	8
Rat07	M	Room B	Throughout	Horse-legs	12
Rat08	F	Room B	Throughout	Horse-legs	7
Rat09*	M	Room B	Throughout	Horse-legs	17
Rat10	F	Room B	Throughout	Horse-legs	5
Rat11	M	Room A	Only at ends	Horse-legs	4
Rat12	F	Room A	Only at ends	Horse-legs	4

Table 1. **Descriptive information for all rats and experimental conditions**

*Did not complete full experimental protocol and thus were excluded from the analysis (see methods)

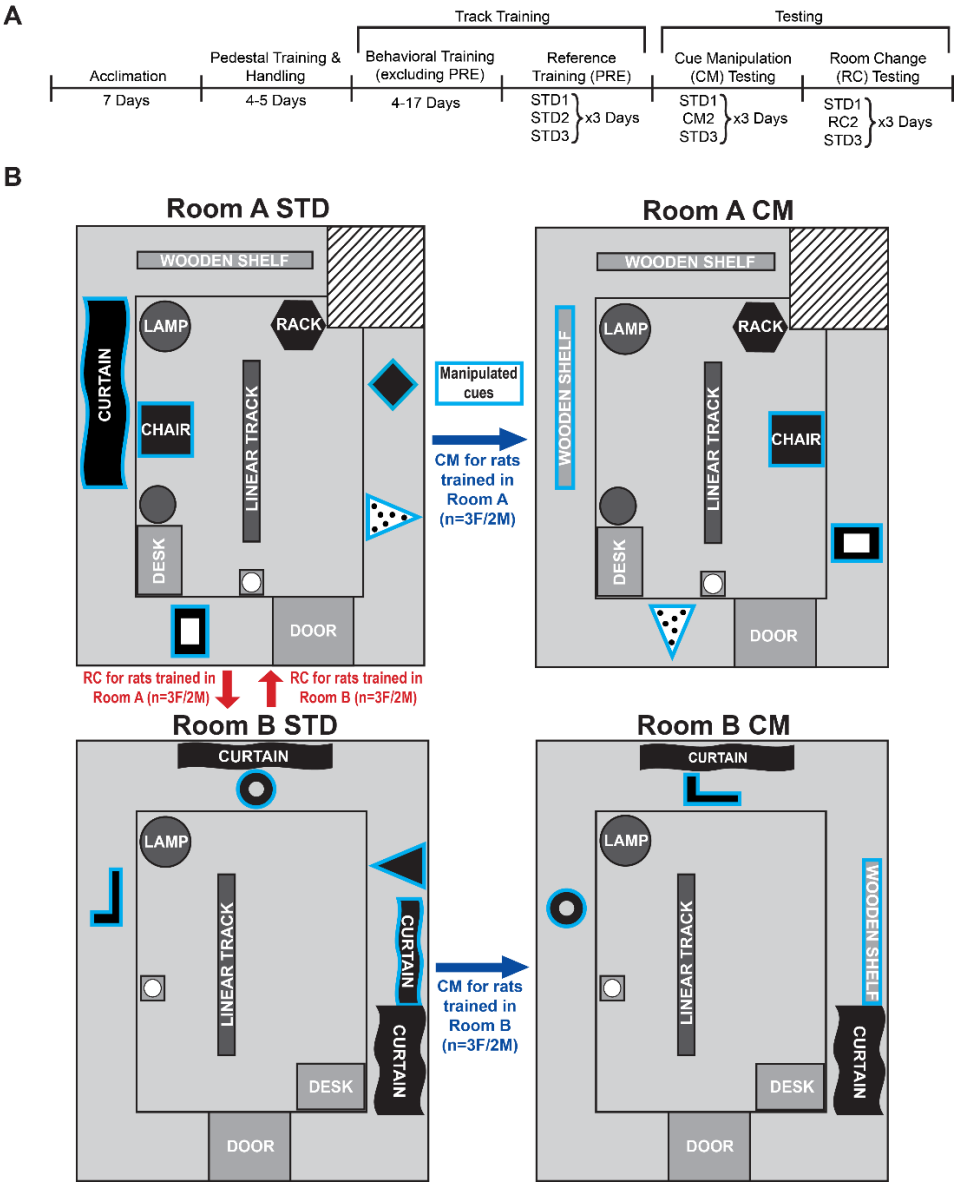

Figure 1. **Experimental timeline, track designs, and cue constellations for all sessions. (A)** Schematic for the experimental timeline for all rats. The duration of track training varied between each animal based on how quickly they mastered the behavioral tasks. **(B)** Schematics of top-down views of room designs and cue constellations for each room and session type. Cues outlined in cyan are manipulated during the cue manipulation (CM) sessions. **(top, left)** Standard (STD) cue configuration for Room A. **(top, right)** CM cue configuration for Room A. The curtain panel shown in Room A STD is lifted to expose wooden shelves underneath it, the chair is moved to the opposite side of the room, the triangle with dots and the rectangular cue are swapped, and the black diamond is removed. **(bottom, left)** STD cue configuration for Room B. **(bottom, right)** CM cue configuration for Room B. Similarly to cue manipulation for Room A CM, a curtain panel shown in the Room B STD cue configuration is lifted to expose wooden shelves, the black L-shaped and black ring cues are swapped, and the solid black triangular cue is removed from the room. For a given animal, all PRE and CM sessions took place in one the room used for training, with its STD and CM configurations respectively, whereas all RC sessions took place in the other room with its STD configuration.

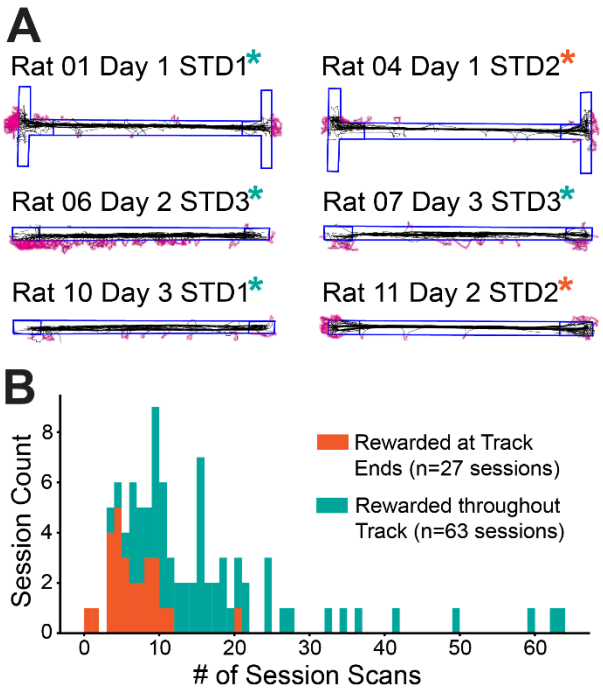

Figure 2. **Off-track scanning in the familiar (training) room and with different food placement patterns. (A)** Six examples of PRE trajectories of animals across all cohorts, and training rooms, shown to highlight the variability in scanning behavior between rats. Track boundaries are outlined in blue, animal trajectories are in black, and scanning events are highlighted in magenta. Rat01 was trained in Room A and rewarded throughout the track, Rats 04 and 11 were also trained in Room A, but were rewarded exclusively at the ends of the track. Rats 06, 07, and 10 were trained in Room B and rewarded all throughout the track (see also Table 1). Asterisks denote the training room (see color code for panel B). Scanning events across the entire track are shown, but scans at the ends of the track are excluded from analysis. **(B)** Stacked histogram of total scans per session for all STD1, STD2, and STD3 PRE sessions by reward location. Teal bars represent the total scans per session for animals rewarded throughout the track, while orange bars represent the animals rewarded exclusively at the ends of the track.

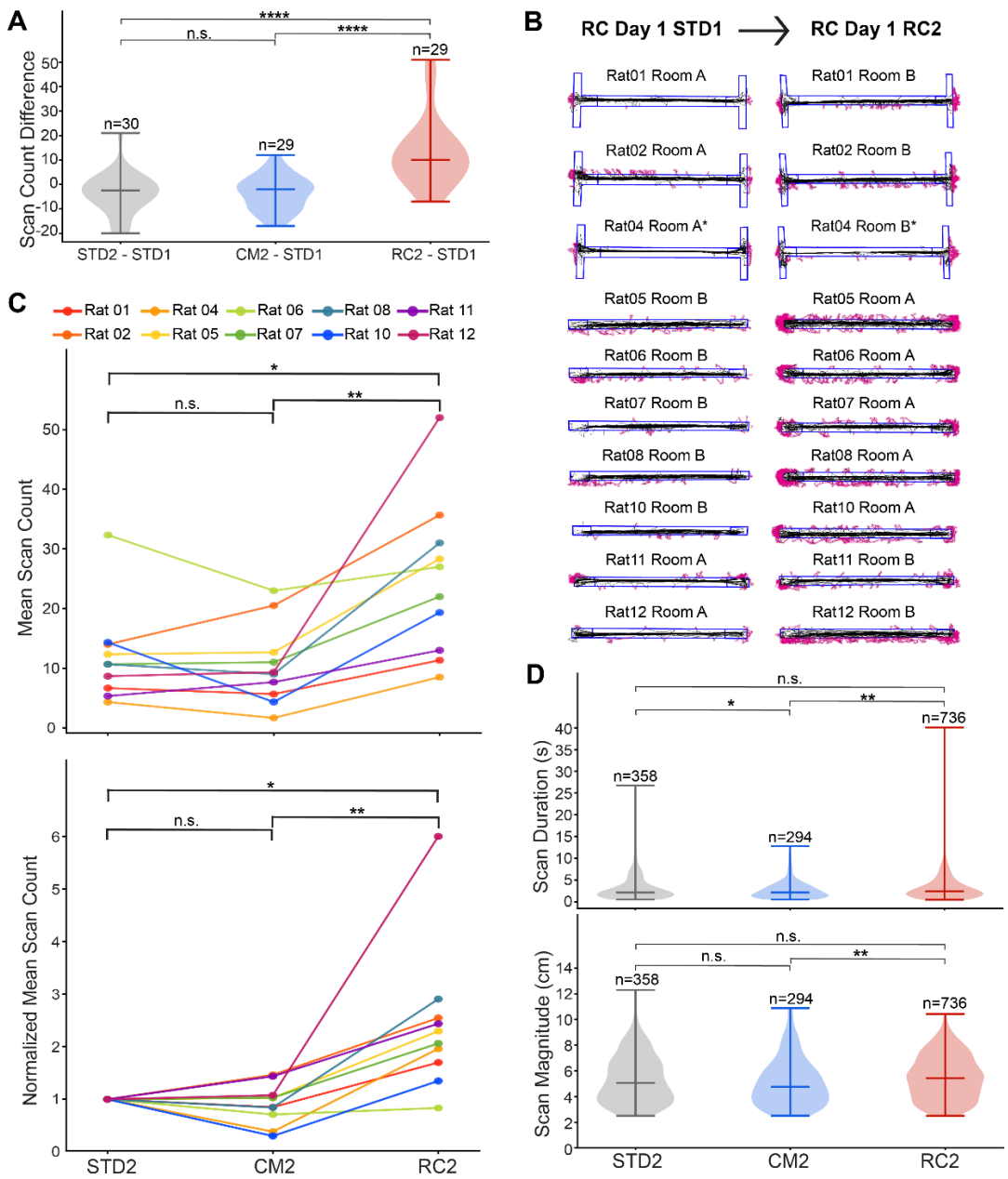

Figure 3. **Off-track scanning across experimental conditions. (A)** Violin plots of scanning increases in STD (familiar), CM (cue manipulation), and RC (room change) conditions. Scan difference is the difference in total scans between the second and first sessions of a session day (i.e., STD2 total scans – STD1 total scans = STD scan difference). **(B)** Example trajectories with scanning events from all 10 rats from the first day of RC experiments. As in Fig. 2, the track boundaries are outlined in blue, animal trajectories are in black, and scanning events are highlighted in magenta. For each pair of examples, the right plot is from the first time the animal was exposed to the novel room, while the left plots show the immediately preceding STD session in the familiar room. **(C)** Paired scatter plots of absolute (top) and normalized (bottom) means of all STD2, CM2, and RC2 experimental sessions, by rat. Scan counts are normalized with respect to the mean scan count of STD2 sessions for each rat in the bottom plot: each rat's mean STD2 score is equal to 1.0000. **(D)** Violin plots of scan duration (top) and magnitude (bottom) across STD2, CM2, and RC2 sessions. **Notes:** *The examples shown from Rat04 are from the second day of the RC experiment, due to technical issues on the day of recording. n.s. p>0.05, *p<=0.05, **p<=0.01, ***p<=0.001, ****p<=0.0001

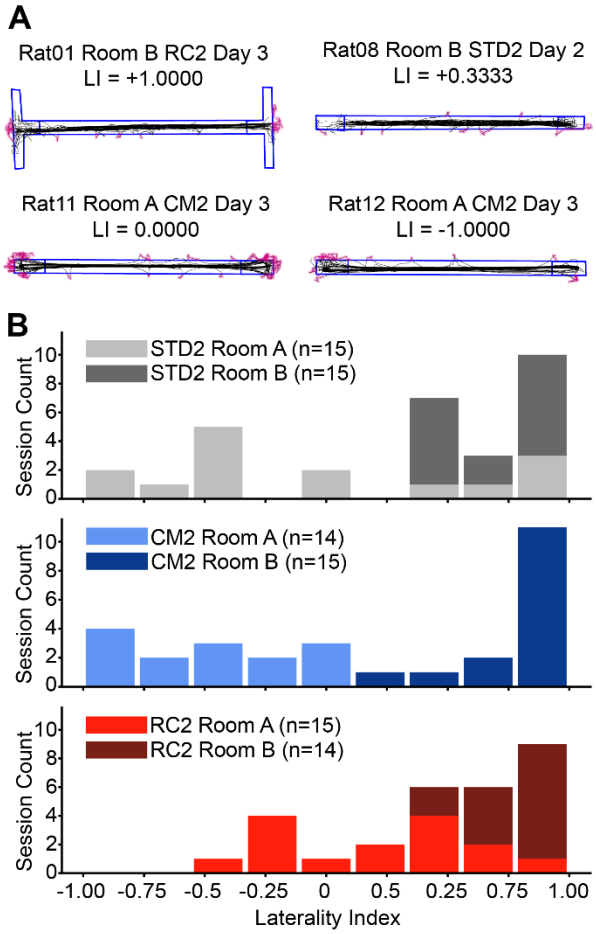

Figure 4. **Lateral biases of off-track scanning behavior. (A)** Example of trajectories displaying varying levels of lateral biases in scanning, labeled with their laterality index (LI). LIs were computed by dividing the count difference of leftward and rightward scans by their sum, such that an LI of -1.0 represents a session in which the rat only scanned leftward scans and an LI of +1.0 represents a session in which the rat only scanned rightward. **(B)** LIs for STD2 (top), CM2 (middle), and RC2 (bottom) sessions stacked by testing room. For STD2 (top; grey) and CM2 (middle; blue) plots, the animals are tested in the same room as their training room, as per PRE and CM protocols. The RC data (bottom; red) is stacked by the room where the RC session was conducted; for example, the animals that completed their CM sessions in Room A are the same animals represented in the RC Room B data shown here.

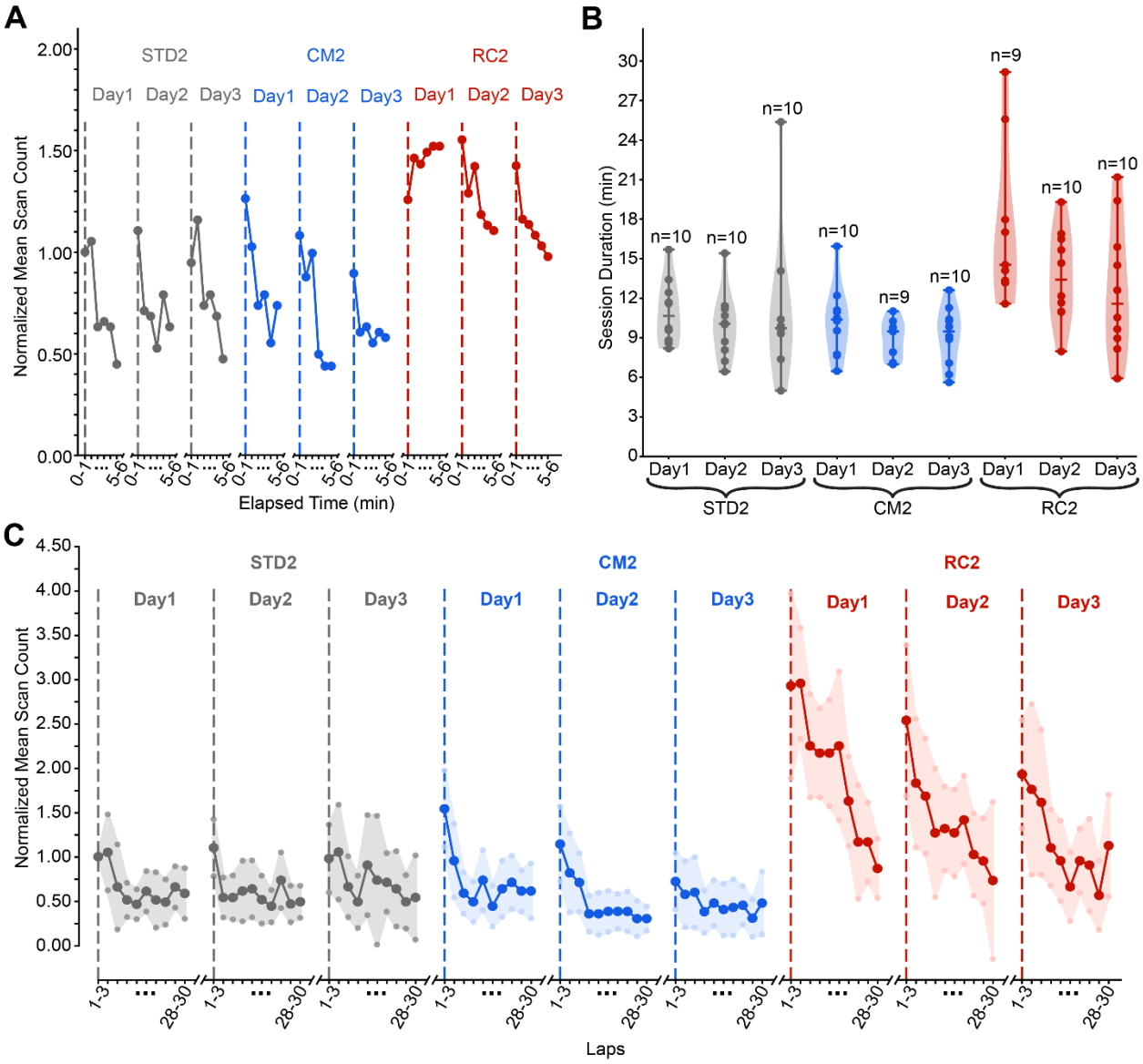

Figure 5. **Evolution of off-track scanning behavior. (A)** Line plot of normalized mean scan counts binned by minute, up to the 6th minute of each session (i.e., truncated based on the time the fastest rats took to run the complete 30-lap sessions). Vertical dashed lines represent the beginning of each new session. **(B)** Durations of all sessions. The dots represent individual session durations for each rat that completed the corresponding session. **(C)** Line plot of normalized mean scan count binned by increments of 3 laps, for all 30 laps. Means are normalized with respect to the mean scan count for the first 3 laps of STD2 Day 1. The shaded region is the 95% confidence interval. Vertical dashed lines represent the beginning of each new session. **Notes:** In these plots and related analyses, scans occurring at the two ends of the track were also included, to avoid gaps in the examined timelines.