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RESEARCH ARTICLE

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Relocating cued goals induces population remapping in CA1 related to memory performance in a two-platform water task in rats

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Abstract

The activity of CA1 neurons in the rodent hippocampus represents multiple aspects of learning episodes, including cue and place information. Previous reports on cue and place representation in CA1 have examined activity in single neurons and population recordings during free exploration of an environment or when actions are directed to either cue or place aspects of memory tasks. To better understand cue and place memory representation in CA1, and how these interact during goal-directed navigation, we investigated population activity in CA1 during memory encoding and retrieval in a novel water task with two visibly distinct platforms, using mRNA for immediate early genes Arc and Homer1a as markers of neural activity. After training, relocating cues to new places induces an extensive, perhaps global, remapping of the memory code that is accompanied by altered navigation and rapid learning of new cue-place information. In addition, we have found a significant relationship between the extent of reactivation and overall cue choice accuracy. These findings demonstrate an important relationship between population remapping in CA1 and memory-guided behavior.

KEYWORDS
hippocampus, IEG, long-term memory, spatial learning, two-platform water task

1 | INTRODUCTION

The CA1 region of the rodent hippocampus encodes multiple aspects of a learning episode, including information about cues and places (Komorowski, Manns, & Eichenbaum, 2009; McKenzie et al., 2014; Muller & Kubie, 1987; Sutherland et al., 2001). Although the hippocampus may not be necessary for acquiring cue memory (McDonald & White, 1993; McDonald & White, 1994; Morris, Haggan, & Rawlins, 1986), and in some cases place memory (Day, Weisand, Sutherland, & Schallert, 1999; Hales et al., 2014; Travis et al., 2010), when the hippocampus is present during a learning episode it is necessary for cue and place memory retrieval (Sutherland, O'Brien, & Lehmann, 2008; Sutherland et al., 2001). Several studies have shown that CA1 place cell activity remaps when cues change location in a familiar spatial context (Knierim, Kudrimoti, & McNaughton, 1995; Lee, Yoganarashimha, Rao, & Knierim, 2004; Muller & Kubie, 1987; Zhang & Manahan-Vaughan, 2015). Specifically, some place cells shift their firing fields in response to cue relocation, while other cells lose their place fields and some begin to exhibit place field activity (Lee et al., 2004; Muller & Kubie, 1987). Previous studies investigating changes in population activity following changes to cue locations have measured unit and population activity while animals freely explore an environment, or while the animal is engaged in distinct cue or place behaviors (Knierim et al., 1995; Leutgeb et al., 2005; McKenzie et al., 2014; Muller & Kubie, 1987; O'Keefe & Nadel, 1978). It remains unclear how changes in CA1 population activity relate to memory performance in goal-directed navigation. Several groups have suggested that CA1 contains a key memory code that is projected to distributed portions of the cortex, and thence utilized for memory-guided behavior (Lee, Zelinski, McDonald, & Sutherland, 2016; Marr, 1971; McNaughton, 2010). Studies on place cell remapping and memory performance have yielded contrasting findings—some groups have reported a relationship between place cell remapping and memory performance (Lenck-Santini, Save, & Poucet, 2001), while others have found no relationship (Jeffery,

Gilbert, Burton, & Strudwick, 2003). It remains possible that remapping across the entire population of CA1 neurons is related to memory-guided behavior.

To address this question, we developed a two-platform water task to induce changes in the CA1 population code and determine how changes in the population code are related to cue choice accuracy (Figure 1a). The two-platform water task requires animals to discriminate between two, visibly distinct platforms (cues) to escape from a pool filled with opaque water (Morris et al., 1986; Sutherland et al., 2001). One of the cues enables escape from the pool throughout training and is supported on a hidden pedestal, while the other cue does not offer escape and is floating in place. Distal room cues are also visible to the animal on the walls surrounding the pool. The positions of the goal cues remain constant relative to the room for an eight-trial session, and on the following eight trials are shifted 90° clockwise or counter-clockwise relative to distal cues (NEW shift), or are shifted 180° (SWITCH shift). If animals express place memory, they are expected to perform better on NEW than SWITCH shifts, due to cue-place conflict on SWITCH shifts (Figure 1a). By contrast, if animals express mostly cue memory, then performance should be equal on NEW and SWITCH cue shifts and choose the correct cue, regardless of its location.

A summary of performance reveals that NEW shifts, especially during early phases of training, induce initially random platform choice, followed by a rapid learning of the correct cue choice (Figure 1b,c). SWITCH shifts result in initial perseveration to navigate toward previously reinforced goal location, which now contains the incorrect cue. As a result, task performance differs in early phases of training when animals are faced with NEW versus SWITCH shifts. Later performance in the two-platform water task is similar on NEW and SWITCH platform shifts, which could suggest a shift from place-controlled to cue-controlled navigation across learning, an observation that is in keeping with previous reports on cue- and place-guided behavior (Morris et al., 1986; Packard & McGaugh, 1996; Tolman, Ritchie, & Kalish, 1946). However, the first cue choice in later training does not reveal a strong preference for the correct cue. It is possible that cue memory has gained associative strength and assists with correct choice during each eight-trial acquisition session.

Navigation during NEW shifts in early phases of the two-platform water task suggests rats have relatively poorer recall of which cue is rewarded and they cannot predict which of the novel locations will be rewarded, and thus the NEW shift is treated as a new learning experience. With SWITCH shifts, rats initially navigate to a previously reinforced location, which contains the incorrect cue, and acquire a new strategy over several trials. We anticipated that a change in the CA1 memory code would be induced by cue shifts in the two-platform water task, and might reflect both new and perseverative navigation strategies in the NEW and SWITCH cue shift conditions, respectively. One method to measure change of the memory code is the amount of similarity in cellular activation that occurs when animals are faced with a NEW or SWITCH cue shifts. To describe population activity that has remained similar, we will use the term "reactivation," and for population activity that has become dissimilar we will use the term "remapping."

We generated two, contrasting hypotheses on the role of remapping and reactivation in the two-platform water task. The first hypothesis was that reactivation would benefit correct cue choice in the two-platform water task, while the second hypothesis was that remapping would benefit correct cue choice. The logic behind our second hypothesis is based on our behavioral results, which might suggest that if cue information does not exhibit strong control over navigation, the same memory will be retrieved before the animal shifts its navigation target in the SWITCH shift condition, followed by a small degree of CA1 remapping when eventually changing strategy after initial perseveration to previous goal locations. By contrast, a NEW cue shift could result in greater CA1 remapping and allow the animal to rapidly implement a new navigation strategy and learn new cue-place information. We expected relocating cues would induce remapping in CA1, and our two hypotheses differ on the proposed role of reactivation versus remapping for performance in the two-platform water task.

To investigate this possibility, we used design-based stereology to examine population activity across the entire septal-temporal axis of CA1 and fluorescent *in situ* hybridization (fISH) to Arc and Homer1a mRNA as markers of neural activity following memory retrieval in the two-platform water task (Figure 2; Schmitz & Hof, 2005; Vazdarjanova & Guzowski, 2004). Our results demonstrate an effect of cue relocation on hippocampal remapping in CA1, and that the extent of similarity across all cue shift conditions is positively related to cue choice accuracy in the two-platform water task. In addition, NEW cue shifts in the two-platform water task induce a significant change in the CA1 memory code, while SWITCH shifts induce a non-significant change in population activity compared to SAME cue-place presentations (Figure 3). This is the first demonstration using the IEG imaging approach, to our knowledge, of a relationship between remapping across the CA1 septal-temporal axis and performance in a memory task.

2 | MATERIALS AND METHODS

2.1 | Subjects

Experimentally naïve, male Long Evans rats weighing between 350 and 400 g (Charles River, Raleigh) were used in each of the present experiments following at least one week of acclimation to the University of Lethbridge animal colony room and 5 days of handling by the experimenter.

2.2 | Two-platform water task acquisition

On the first day of two-platform water task acquisition rats were brought into a room containing a fiber glass swimming pool (2.0 m diameter) filled with room temperature water (~21°C) and several distal cues surrounding the pool (Figure 1a). Two visible platforms (cues) with different appearances (one solid black with a rubber lining; the other painted with black and white stripes on PVC imitation wood) located in the center of opposite quadrants in the pool, ~2 inches above the water surface. One of the cues was supported with a hidden pedestal for a given rat throughout training and testing (reinforced cue), while

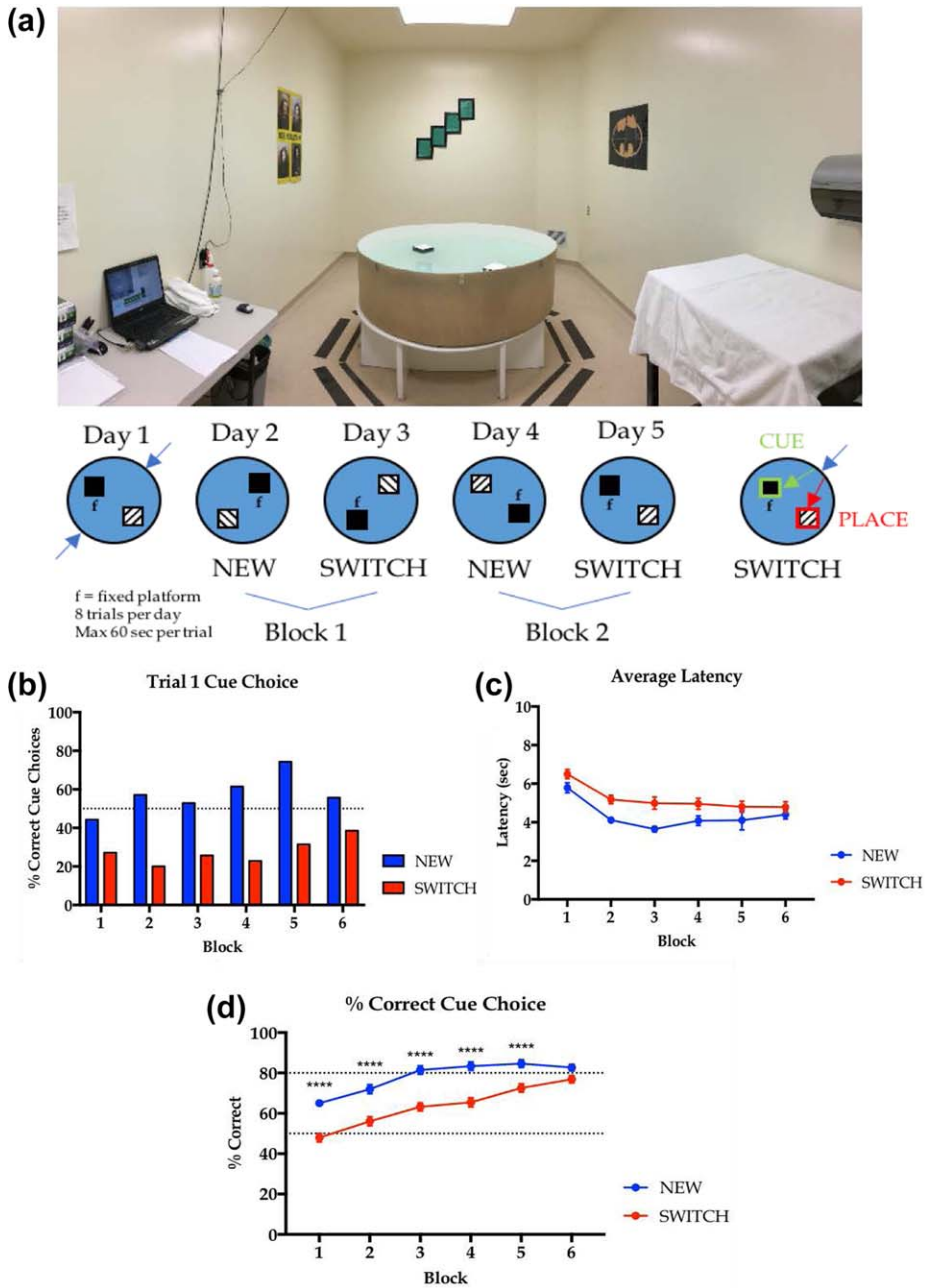

FIGURE 1 Behavioural setup and performance summary of two-platform water task acquisition. (a) Two-platform water task room arrangement and schematic depiction of task design. Training in the two-platform water task alternates between NEW (90°) and SWITCH (180°) cue shifts in a pool filled with opaque room temperature water. One of two visbly distinct platforms (cues) is supported throughout training using a hidden pedistal, while the other is tethered and floating in a stable position. The control of cue and place strategies on navigation are revealed following SWITCH cue shifts when animals are faced with a conflict between a previously reinforced place that is occupied by the incorrect cue (lower panel). (b) Trial 1 percent correct cue choice following NEW and SWITCH cue shifts. The data summary reveals that rats choose the incorrect cue (below chance) that occupies the previously correct place during early phases of two-platform water task training following SWTICH cue shifts, suggesting that place information controls behavior during earlier phases of two-platform water task acquisition. However, a summary of the correct cue choice also suggest that animals do acquire cue memory that assists performance on NEW cue shifts over each eight trial session. (c) Two-platform water task acquisition percent correct cue choice during NEW and SWITCH cue shifts from each eight-trial session. Performance shows a clear division over the eight trial sessions following NEW and SWITCH cue shifts, resulting greater percent correct cue choice in the NEW compared to SWITCH shift condition. This supports that place information controls memory-guided behavior in early task acquisition, and later performance becomes similar in both NEW and SWITCH cue shifts, possibly due to cue memory acquiring greater associative strength (block 6). (d) Two-platform water task acquisition average latency to the correct cue following NEW and SWITCH cue shifts. A summary of average latency to the correct cue during each eight-trial session reveals a similar pattern as in (c), showing that animals take longer to navigate to the correct cue following SWITCH compared to NEW cue shifts in the two-platform water task[Color figure can be viewed at wileyonlinelibrary.com]

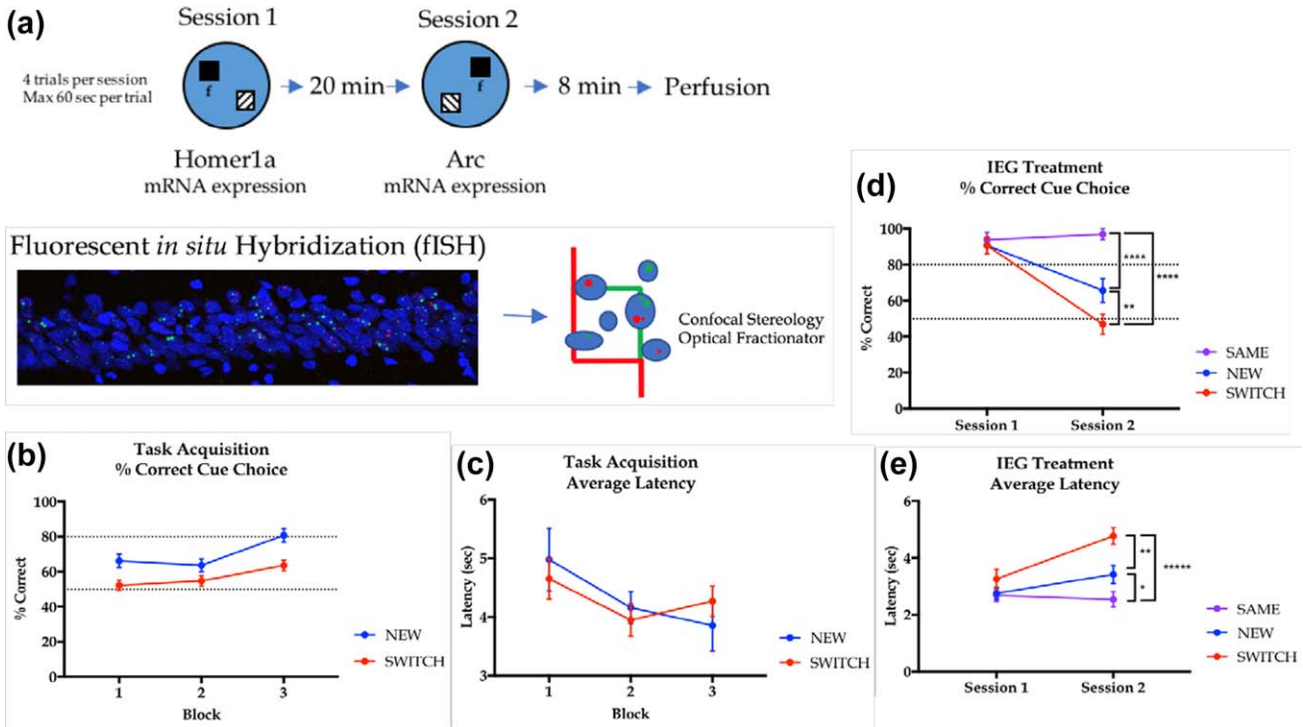

FIGURE 2 IEG Activation task and imaging design and behavioural performance. (a) Schematic diagram of IEG Activation task design and example image of a CA1 confocal z-stack of fISH-processed tissue. In session 1 animals swam four trials with cues in the same position as a previous session to activate Homer1a mRNA expression. This was followed by a 20-min return to the home cage and then a second, four-trial session in which the cues were shifted to NEW and SWITCH arrangements, or not shifted at all in the SAME group. Rats were then perfused and had their brains processed for fISH staining. Folloing fISH tissue processing, DAPI, Homer1a, Arc, and double label markers were estimated using the optical fractionator method adapted for confocal stereology. (b) Two-platform water task acquisition percent correct cue choice. The results from the second cohort of animals used for IEG Activation and quantification displayed similar behavior in percent correct cue choice as animals that performed the extended task in the data summary (Figure 1). Correct cue choice was greater following NEW than SWITCH cue shifts across the three acquisition blocks prior to IEG activation. (c) Two-platform water task average latency to the correct cue. The cohort used for IEG Activation and quantification did not display a reliable difference in NEW compared to SWITCH average latency to the correct cue during task acquisition, unlike animals in the data summary. This difference in results across the present experiments suggests that percent correct cue choice is a more sensitive measure to detect differences in navigation strategy in the two-platform water task. (d) IEG Activation percent correct cue choice. Performance in the SAME group in session 1 and 2 suggest that when cues occupy the same location as the previous session, rats are able to reliably retrieve the correct cue-place memory. However, following a NEW cue shift, there is a drop in session 2 performance due to initially random choice when the cues occupy new places, followed by rapid learning of the correct cue-place strategy. Finally, SWITCH cue shifts during IEG Activation resulted in animals persisting to target the incorrect cue in the previously correct place, causing a greater decline in percent correct cue choice during session 2. (e) IEG Activation average latency to the correct cue. In keeping with percent correct cue choice during IEG Activation, rats were able to quickly navigate to the correct cue in the SAME cue shift condition during session 1 and 2. Differences in average latency performance are evident during session 2, when animals take longer to reach the correct cue during NEW and SWITCH cue shifts due to incorrect cue choices, with the greatest latency to reach the correct cue following a SWITCH cue shift [Color figure can be viewed at wileyonlinelibrary.com]

the other cue was floating in place (non-reinforced) and tethered to the bottom of the pool such that it would sink if the animal attempted to escape the pool using the cue. The animal was carefully placed in the water facing the pool wall at one of two locations equidistant from either cue and allowed to swim for a maximum of 60 s per trial with a 10-s timeout following each trial. If the rat did not reach the correct cue by the end of the trial it was placed on the correct platform for 10 s before returning to its holding cage. The cage was also covered with a bath towel to prevent the animal from viewing its surrounding between trials. Each animal swam a total of eight trials per day with between two and four minutes between trials before returning to its home cage for 24 hr. Importantly, given the stable cue contingency and location on a given day, rats could use either a cue or place strategy to navigate to the correct cue. Egocentric strategies (turning response) cannot be used to successfully navigate since starting locations from opposite quadrants of the pool would not be associated with reinforcement of a specific turning response. Thus, manipulations were made of the platform locations to determine which strategy, either cue or place, controlled the animals' behavior across training. On the following day, the cue contingencies were kept the same for each animal, and both cues were rotated 90° in the pool with respect to the distal cues either clockwise or counter-clockwise (NEW shift). If rats demonstrate a

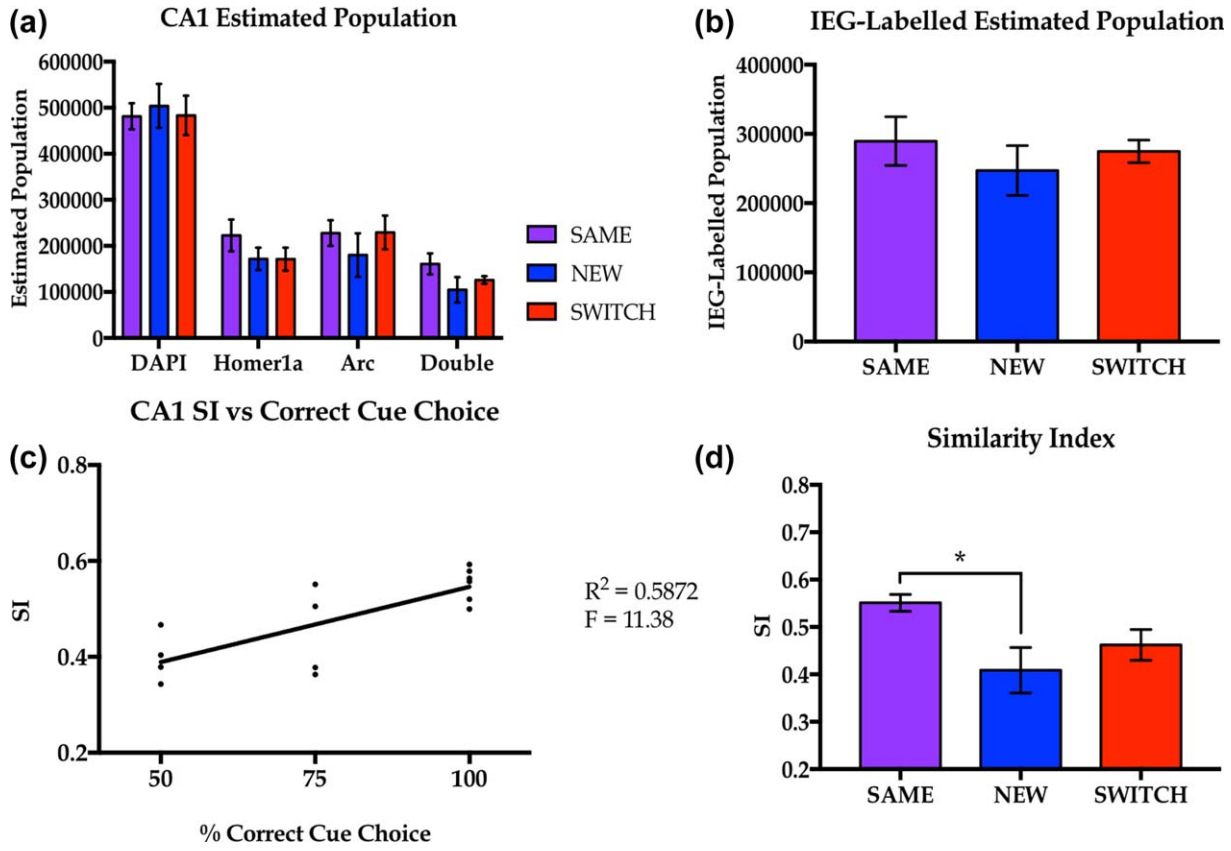

FIGURE 3 IEG quantification results. (a) CA1 estimated population for all markers and IEG Activation groups. Results from stereologic quantification of DAPI and IEG markers showed a significant effect of label but not group or label × group interaction. (b) IEG-labeled estimated population. Following calculation of the total number of cells expressing IEG labels (see Section 2) we compared the estimated population of IEG-labeled cells in CA1 across IEG activation groups. A one-way ANOVA revealed no main effect of group on the total population of labeled cells in CA1. (c) Linear regression of SI and percent correct cue choice in session 2 of IEG activation. We performed a linear regression to examine the relationship between SI as a measure of the extent of CA1 population remapping and percent correct cue choice. Our results demonstrate a significant positive correlation between these measures, suggesting that greater SI results in better performance in the two-platform water task. (d) SI following different cue shifts in IEG Activation. Using SI as a measure of the extent of remapping across the CA1 population, we found a significant effect of cue shift on SI. Post hoc comparisons revealed that SI was significantly lower following a NEW but not SWITCH cue shift compared to the SAME shift condition[Color figure can be viewed at wileyonlinelibrary.com]

strong cue response they should make correct cue choices on the first trial of the NEW shift. Alternatively, if rats do not have a strong cue memory they might make a random cue choice initially, followed by re-acquisition of the correct cue-place strategy. The difference between cue and place control over the rats' navigation is illustrated on the following day when the animal is returned to the room with the platforms rotated 180° relative to the distal cues from the previous day of training (SWITCH shift). If animals maintain a strong cue strategy, they would choose the correct cue on the first trial and thereafter. However, if they express a strong place strategy they will choose the non-reinforced cue for several trials before correcting their navigation to the correct cue in the opposite location relative to the previous day of training. If animals possess a correct cue representation and place representation they might make an incorrect choice initially and, depending on the associative strength of each aspect, navigate to the correct cue sooner or later in the trials on that day. Each pair of NEW and SWITCH shifts are considered as a single block of training, and each rat experiences the NEW and then a SWITCH shift during a training block. Initial behavioral assessment of task acquisition was carried out for at least six blocks of training (15 days) whereupon performance on latency and percent correct cue choice across the NEW and SWITCH sessions became statistically equal across the eight trials of swimming. For IEG treatment, acquisition ended following three blocks of training (7 days) when performance tended to rise above an 80% threshold upon a NEW cue shift.

2.3 | IEG activation

Following completion of three acquisition blocks in the two-platform water task, rats were given one of three IEG activation treatments to probe neural activity dynamics following different cue shifts. In each condition, rats returned to the room ~24 h after the third block of training with the platforms in the same position as the previous day and were given four swim trials (1-min inter-trial interval; total 5-min session) to assess memory and re-activate the neural ensemble representing the previous cue arrangement. The first four trials of swimming, referred to as "session 1", drive the expression of Homer1a mRNA as a marker of neural activity. Following the completion of session 1, rats

were brought back to their home cages for 20 min. Thereafter, rats were given one of three cue manipulations in the following four trials referred to as "session 2". In the SAME condition, rats were returned to the room and swam for four trials (1-min inter-trial interval; total 5-min session) with the cues in the same position as the previous four trials. By contrast, in the NEW condition rats were returned to the room and swam for four trials with the platforms rotated 90° clockwise or counter-clockwise relative to session 1, and in the SWITCH condition the rats swam for four trials with the platforms rotated 180° relative to session 1. The second session was used to drive the expression of Arc mRNA as a marker of neural activity during each cue manipulation. 90 s following the fourth swim during session 2 rats were given a 1.5 ml intraparitoneal injection of sodium pentobarbital and transported to a separate room for perfusion and tissue collection.

2.4 | Animal perfusion and tissue collection

Approximately eight minutes following session 2 of the IEG activation rats were perfused intracardially with 100 ml of cold 1× phosphate-buffered saline and diethyl pyrocarbonate (PBS-DEPC) solution followed by 100 ml of 4% paraformaldehyde (PFA) dissolved in 1× PBS-DEPC solution. The brain was immediately removed from the skull and kept at 4°C overnight in 4% PFA in 1× PBS-DEPC solution, and then transferred to 30% sucrose dissolved in 1× PBS-DEPC solution for at least 48 hr prior to sectioning. Before cryosectioning each brain was hemisectioned sagittally down the midline with a sterilized razor blade and then sliced at 40 μm thickness throughout the entire extent of the hippocampus. Every 12th section was collected and mounted on Superfost Plus (Fisher Scientific) ionized slides for fluorescent *in situ* hybridization (fISH) tissue processing and quantification of IEG expression.

2.5 | fISH tissue processing

Primers flanking portions of Arc intron 1, exon 2 and intron 2 were designed using online software (National Center for Biotechnology Information Primer-Blast). The exact sequences of the primers are as follows and base pair designations match those of GenBank accession number NC_005106: 5′-CTTAGAGTTGGGGGAGGGCAGCAG-3′ (forward primer, base pairs 2022–2045) and 5′-ATTAACCCTCACTAAAGGG-CCCTGGGGCCTGTCAGATAGCC-3′ (reverse primer tagged with T3 polymerase binding site on 5′ end, base pairs 2445–2466). Polymerase chain reaction (PCR) was performed on genomic rat DNA template using a Taq PCR Kit (New England Biolabs, Ipswich, Massachusetts, USA) and the PCR product was purified using a Qiagen PCR Purification Kit (Life Technologies Inc., Carlsbad, California, USA). A commercial transcription kit (MAXIscript T3; (Life Technologies Inc., Carlsbad, California, USA) and Digoxigenin (DIG) RNA Labeling Mix (Roche Diagnostics, Risch-Rotkreuz, Switzerland) were used to generate DIG-labeled Arc intron-specific antisense riboprobes from the PCR template. Fluorescein-labeled Homer1a probes targeting the 3′ untranslated region were generated as previously described (Montes-Rodríguez et al., 2013). Riboprobes were purified with mini QuickSpin columns (Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Fluorescent in situ hybridization was performed as described by Montes-Rodríguez et al., (2013). Briefly, DIG-labeled Arc riboprobe signal was amplified with anti-digoxigenin-POD (1:300; Roche Diagnostics), tyramide signal amplification (TSA) Biotin Tyramide Reagent Pack (1:100; PerkinElmer) and Streptavidin-Texas Red (1:200; Perkin Elmer). Fluorescein-labeled Homer1a probe was detected with anti-Fluorescein-HRP antibody (1:1000; Jackson ImmunoResearch Labs) and amplified with a Fluorescein TSA kit (1:100; PerkinElmer). Nuclei were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI; 1:2000; Sigma-Aldrich).

2.6 | CA1 IEG quantification

IEG expression was quantified using the optical fractionator method in StereoInvestigator software (version 10.54) from confocal z-stack images collected on an Olympus FV1000 equipped with Fluoview FV10-ASW software (version 4.0). Unilateral traces of CA1 were placed over live images at 20× objective on each section prior to z-stack image acquisition. The counting frames were positioned on a 150 × 150 μm grid over the CA1 trace according to principles of systematic-random sampling. A series of seven z-stack images at 512 × 512 pixels were collected at each sampling site with a 60× oil objective starting at the top of the section every 2 μm for a total 14 μm stack. Image thresholds were set at 720 HV ± 20, 600 HV ± 20, and 575 HV ± 20 respectively in DAPI, FITC, and Texas Red channels and kept constant across imaging a section series such that small Homer1a and Arc transcription foci (2–3 pixels in diameter) could be clearly identified. Z-stack images were imported into StereoInvestigator such that one image from each stack fell above and another below the 10-μm dissector height. DAPI was counted according to optical dissector inclusion–exclusion criteria at each cell's widest point. If included cells contained Homer1a, Arc, or Double Labels, each were counted individually using separate markers.

2.7 | Data analysis

Statistical analyses were performed using SPSS (Version 21.0, IBM, Armok, New York, USA), G*Power (Düsseldorf, Germany), and Prism by GraphPad (San Diego, California, USA) software. Behavioral data from percent correct cue choice and latency to the correct cue in SAME, NEW, and SWITCH cue conditions were analyzed using a mixed-model ANOVA with block and cue shift as factors. Post-hoc LSD pairwise comparisons were performed following significant block X cue shift interaction, comparing performance in cue shift conditions on individual blocks. Initial analyses for effects in imaging data were performed using a mixed-model ANOVA on stereologic estimates of DAPI, Homer1a, Arc, and Double Label marker averages with label and group as factors. Total number of labeled cells was computed and compared across groups to examine a main effect of group on IEG-labeled CA1 cells. The proportion of double labeled cells out of the total labeled population, referred to as similarity index (SI), was calculated for each animal and average SI was compared across groups using a one-way ANOVA. Post-hoc uncorrected LSD comparisons were performed following a

significant effect of group on SI. The number of total labeled cells and SI were calculated for each animal using the following equations:

$$Q_{Tot} = (Q_{H1a} + Q_{Arc}) - Q_{Dbl}$$

$$SI = Q_{Dbl}/Q_{Tot}$$

Thus, a SI value of 1 would indicate absolute similarity in Homer1a and Arc IEG expression, whereas a SI value of 0 would indicate absolute orthogonality in the population.

3 | RESULTS

3.1 | Two-platform water task acquisition

A summary of control animal performance ($n = 72$) in the two-platform water task revealed that rats acquire the correct cue strategy sooner on NEW than SWITCH cue shifts. We found a robust effect of cue shift ($F(1,71) = 134.4$, $p < .0001$), block ($F(5, 355) = 55.41$, $p < .0001$), and a significant shift X block interaction ($F(5,355) = 2.775$, $p = .0179$) on percent correct cue choice (Figure 1c). In latency to the reach the correct cue, we also found a significant effect of cue shift ($F(1,71) = 75.71$, $p < .0001$) and block ($F(5,355) = 16.41$, $p < .0001$), but not a significant shift X block interaction ($F(5,355) = 1.145$, $p = .3364$; Figure 1d). Trial 1 cue choice also reveals that animals make initial cue choices at a chance level during the first three blocks of acquisition on NEW cue shifts (Figure 1b). Later in training, some rats improve in their immediate retrieval of the correct cue during NEW cue shifts on the first trial, although the cue choice does not appear to be greater than chance in block 6. As mentioned previously, cue information may gain some associative strength to assist in better overall performance across the eight trials during NEW shifts. By contrast, SWITCH cue shifts result in rats choosing the incorrect platform in the previously correct place, indicating that rats retrieve the previously reinforced correct cue location in early two-platform water task acquisition. The robust differences between correct cue choice and latency during two-platform water task suggest that, although animals might use visual cues to guide navigation following three blocks of training, place memory maintains strong control on navigation until performance becomes similar in later blocks of two-platform water task acquisition.

In a separate cohort of animals used to probe IEG expression ($n = 24$) we replicated the effects of two-platform water task acquisition in cue choice over three blocks of training in cue shift ($F(1,23) = 19.46$, $p = .0002$) and block ($F(2,46) = 21.21$, $p < .0001$) prior to IEG treatment, and no significant shift × block interaction ($F(2,46) = 0.7805$, $p = .4642$; Figure 2b). Similar effects of cue shift, block, and shift × block interaction occur if only the first three blocks of data are considered from the summary data, above ($F(Shift(1,71)) = 39.33$, $p < .0001$; $F(Block(2,142)) = 56.61$, $p < .0001$; $F(Shift \times Block(2,142)) = 0.9267$, $p = .3982$). Notably, we found a significant effect of block ($F(2,46) = 4.116$, $p = .0227$) but no significant effect of shift ($F(1,23) = 0.0148$, $p = .9042$) and no significant shift × block interaction ($F(2,46) = 0.6338$, $p = .5351$) in latency to the correct cue in this cohort during acquisition (Figure 2c), suggesting that percent correct cue choice is a more sensitive measure to detecting performance changes following cue shifts. After three blocks of two-platform water task acquisition, we sought to examine neural activity dynamics using the IEGs Arc and Homer1a as markers of neural activity following SAME, NEW, or SWITCH cue shifts.

3.2 | IEG activation

The IEGs were activated in two, four-trial swim sessions separated by twenty minutes (Figure 2a). This design allows us to assess Homer1a mRNA expression as a marker of neural activity during the first session, and Arc mRNA expression as a marker of neural activity during the second session. During the first session rats were returned to the room with the cues in the same position as the previous day of training, and were given four swim trials with a one-minute inter-trial interval over a five-minute session. The rats were then returned to their home cage for twenty minutes before coming back to the room with the cues shifted to one of three possible locations: SAME (0° shift), NEW (90° shift), or SWITCH (180° shift). The rats swam for an additional four trials with 1-min inter-trial intervals over a 5-min session in one of the three shift conditions and were then perfused and had their brains extracted ~8 min after the second session.

Behavioral results from this phase of the task illustrate that each group in the SAME, NEW, and SWITCH cue shift conditions successfully retrieved the correct cue-place strategy during session 1 (Figure 2d). Performance in session 2 varied across shift conditions, resulting in a significant effect of session ($F(1,21) = 26.84$, $p < .0001$), shift ($F(2,21) = 17.15$; $p < .0001$), and session × shift interaction ($F(2,21) = 10.41$; $p = .0007$; Figure 2d). Although uncorrected post-hoc LSD comparisons revealed no significant differences in percent correct cue choice in session 1, there were significant differences in percent correct cue choice between SAME versus NEW ($p < .0001$), SAME versus SWITCH ($p < .0001$), and NEW versus SWITCH ($p = .0097$) conditions during session 2. We found similar effects in latency to the correct cue, resulting in a significant effect of shift ($F(2,21) = 9.338$, $p = .0003$), session ($F(1,21) = 9.338$, $p = .006$), and shift × session interaction ($F(2,21) = 4.642$, $p = .0214$; Figure 2e). In addition, we found significant differences between SAME versus NEW ($p = .0304$), SAME versus SWITCH ($p < .0001$), and NEW versus SWITCH ($p = .0014$) cue shifts in latency to the correct cue in session 2, but no significant differences between shift conditions in session 1. These findings extend the results of the two-platform water task summary in both groups and further show that rats can maintain a reliable memory of the correct cue-place strategy in the SAME cue condition, are able to rapidly encode a new cue-place strategy in the NEW condition, and perform significantly worse following SWITCH cue shifts due to navigation to the incorrect cue for several trials. We anticipated that the CA1 population would remain stable in the SAME condition, given the accurate performance in both sessions 1 and 2. In general, we expected that cue relocation would cause CA1 remapping following a NEW or SWITCH cue shift. However, SWITCH cue shifts might cause less remapping due to different cues occupying the same locations, while NEW cue shifts might induce greater remapping. Our first hypothesis suggests that reactivation (higher similarity) should benefit performance across all groups,

while the second hypothesis suggests that remapping (lower similarity) should benefit performance following shifts.

3.3 | CA1 IEG expression

Following Arc and Homer1a mRNA labeling, we estimated the population of DAPI, Homer1a, Arc, and Double Labels across the septal-temporal axis of CA1 using a confocal design-based stereology approach in a randomly chosen, representative subset of animals from the behavioral cohort ($n = 14$; Figure 2a). These animals did not differ in their behavior from the greater cohort during session 2 of IEG activation ($F(1, 32) = 2.564$; $p = .1192$). Our results indicate a similar number of DAPI-labeled cells in a single hemisphere of CA1 to previous reports using similar methods (Heggland, Storkaas, Soligard, Kobro-Flatmoen, & Witter, 2015), suggesting that the present confocal design-based stereology approach provides a reliable estimation of cell number (Figure 3a). We found a significant effect of label ($F(3,33) = 91.73$, $p < .001$) in our population estimates, but not a significant effect of group ($F(2,11) = 0.6531$, $p = .5395$)) or label × group interaction ($F(6,33) = 0.5856$, $p = .7392$; Figure 3a). We normalized the active population of neurons in each animal using the simple calculation: $Q_{Tot} = (Q_{H1a} + Q_{Arc}) - Q_{Dbl}$. A one-way ANOVA showed no significant effect of group on the estimated number of labeled CA1 neurons ($F(2,11) = 0.6383$, $p = .5467$; Figure 3b). Following normalization, we sought to determine how similar the population of active neurons was between sessions 1 and 2 in each group using a similarity index (SI) measure. To determine SI we used the following calculation for each animal: $SI = Q_{Dbl}/Q_{Tot}$. Thus, SI measures the proportion of cells labeled in both sessions out of the total population of labelled cells, without assuming any pattern of recruitment to the active population (Witharana et al., 2016). We first examined the relationship between SI and performance during session 2 of IEG treatment to answer if there was a significant relationship between reactivation or remapping and memory retrieval at the behavioral level. A linear regression of SI versus percent correct cue choice in session 2 on all groups revealed a strong correlation between memory reactivation measured with SI and performance of correct cue choice ($R^2 = .5858$, $F = 16.97$, $p = .0014$; Figure 3c). When we performed a follow-up regression on animals from the NEW and SWITCH shift groups only we found a trending but non-significant positive correlation between SI and percent correct cue choice ($R^2 = .3556$, $F = 3.863$, $p = .09$). We then sought to further test our prediction that cue shifts in the two-platform water task during session 2 would result in remapping. A one-way ANOVA showed a significant effect of group ($F = 4.694$, $p = .0336$, $\eta_p^2 = 0.60$; Figure 3d), confirming that cue shifts induce a significant change in the CA1 population code. Uncorrected LSD post-hoc comparisons revealed that NEW ($n = 4$; $p = .0122$, $d = 2.10$) cue shifts caused a significantly lower SI score compared to the SAME cue condition ($n = 5$), while SWITCH shifts resulted in a trending but not significantly lower SI ($n = 5$; $p = .0731$, $d = 0.60$). We did not find a significant difference between NEW and SWITCH cue shift groups ($p = .2837$, $d = 0.64$). Together, these results demonstrate a positive relationship between cue choice accuracy and CA1 remapping, and that remapping might have different functions when animals are faced with SAME, NEW, or SWITCH cue shifts.

4 | DISCUSSION

Our findings demonstrate an important relationship between the extent of CA1 population remapping and memory-guided navigation. We have found a significant correlation between ensemble reactivation and memory retrieval in a two-platform water task, and that relocating cued goals in induces remapping in CA1 related to the learning of new cue-place information. This finding supports our first hypothesis that reactivation benefits correct cue choice in the two-platform water task. This is the first demonstration, to our knowledge, of a significant relationship between ensemble reactivation across the septal-temporal axis of CA1 and memory retrieval using the IEG method. However, it may also be the case that remapping has a distinct function following cue shifts. NEW cue shifts may result in immediate remapping with initially random cue choice, followed by rapid cue-place learning; SWITCH shifts may result in retrieval of a more similar memory due to cues locating the same positions with worse overall performance due to retrieval of previous place associations. We view this as the most consilient explanation of our behavioral data, although more investigation is clearly needed. We have found a significant difference in SI between groups subjected to SAME and NEW cue shifts, but not between SAME and SWITCH cue shifts. However, we did not find a significant difference between NEW and SWITCH cue shifts. Based on our findings, we cannot rule out another explanation, that remapping could have different functions following SAME, NEW, or SWITCH cue shifts. Importantly, our results support the idea that cue relocation induces population remapping in CA1 and that similarity in the memory code is positively related to cue choice accuracy in the two-platform water task. These findings also add to a growing literature describing the representation of multiple aspects of long-term memory in the rodent hippocampus and its relevance to animal behavior.

Based upon retrograde amnesia effects, a surprisingly broad range of aspects in a learning episode are represented in the rodent hippocampus (Lee et al., 2016; McKenzie et al., 2014; Wood, Dudchenko, Robitsek, & Eichenbaum, 2000). Hippocampal disruption using either temporary inactivation or permanent lesions causes robust retrograde amnesia for context fear (Gulbrandsen, Sparks, & Sutherland, 2013; Sutherland et al., 2008; Sutherland, Sparks, & Lehmann, 2010), context discrimination (Lee, Sutherland, & McDonald, 2017), tone fear (Sutherland et al., 2008), fear-potentiated startle (Lehmann, Sparks, O'Brien, McDonald, & Sutherland, 2010), cue memory (Sutherland et al., 2001), picture memory (Epp et al., 2008), home base memory (Travis et al., 2010), spatial memory (Broadbent, Squire, & Clark, 2004; Sutherland et al., 2001), and episodic memory (Steinvorth, Levine, & Corkin, 2005). In a recent review we discussed these findings and their implications for a new view on the role of the hippocampus in long-term memory (Lee et al., 2016). We proposed a new concept, termed heterarchic reinstatement (HR), to account for a broad range of these results. On this view, the output of activity from the hippocampus to the cortex during a learning episode will result in the hippocampal output to the cortex becoming an essential part of most or all target memories. The HR concept predicts that changes in the output of the hippocampus to the cortex will result in changes to the target memory, and task behavior. Thus, HR suggests that population remapping

would result in changes at the behavioral level for the many aspects of memory encoded in CA1 cell activity.

Several reports have described that many features of a learning episode are encoded in single-cell and population activity in CA1, including place, visual cues, odors, approach behaviour, and anticipated rewards (Komorowski et al., 2009; McKenzie et al., 2014; Wood et al., 2000). However, some authors have recently questioned whether simple cues represented in hippocampal activity are necessary for guiding animal behavior (Ainge, Tamosiunaite, Wörgötter, & Dudchenko, 2012). For example, Ainge et al. (Ainge et al., 2012) described that place unit activity is not controlled by discriminative visual cues, but instead is under control of the animal's goal location. By contrast, McKenzie et al. (McKenzie et al., 2014) found that place field firing rates can be modified by repeated presentations of a cue in a context-specific location followed by reward. In the current study, we have found that changes in the CA1 memory code are related to changes in visual cue discriminations. Notably, we have examined this relationship following just three blocks of training when spatial memory also has strong control over behavior. It would be interesting in future studies to examine if the relationship between remapping and correct cue choice remains following additional training when animals make responses that may be more strongly controlled by cues.

Previous studies on place cell remapping in the hippocampus have revealed that CA1 has distinct remapping characteristics from the dentate gyrus (DG) and CA3 (Lee et al., 2004; Leutgeb, Leutgeb, Moser, & Moser, 2007; Leutgeb et al., 2005; Vazdarjanova & Guzowski, 2004). While CA1 tends to show continuous place cell remapping in response to changes in spatial context, CA3 exhibits discontinuous or attractor-like remapping, and the dentate gyrus tends to show remapping following minor changes in spatial context (Lee et al., 2004; Leutgeb et al., 2007). In future studies, it will be important to examine the relationship between remapping in CA3 and the DG to changes in memory-guided behavior. We anticipate that the changes in population activity in the DG-CA3 circuit is the cause of remapping in CA1, and that pattern separation processes may be critical to recognizing shifts in cue orientation relative to previous experience in the two-platform water task and the rapid learning of new cue-place information. Although pattern separation may be a general computation also shared by cortical networks (Leutgeb & Leutgeb, 2007; Yassa & Stark, 2011), the hippocampal circuit likely provides a unique contribution in its ability to rapidly retrieve a target memory and detect when a spatial context has changed.

The present findings are the first demonstration, to our knowledge, of a significant relationship between cellular reactivation and memory retrieval at the behavioral level applying the IEG imaging approach across the entire CA1 septal-temporal axis. Importantly, we have found that this relationship is robust in a cued navigation task with a simple visual discrimination guiding behavior. In combination with other studies on changes in the memory code and its relation to behavior (Danielson et al., 2016; Dupret, Pleydell-Bouverie, & Csicsvari, 2010; Komorowski et al., 2009; McKenzie et al., 2014), these data suggest that multiple features represented in CA1 activity make an important contribution to memory retrieval. In future studies, it will be important to characterize which representations at the single-unit and population level maintain a significant relationship to memory behavior across training in the two-platform water task or a similar task, and are affected by changes to cue-place presentation in a spatial context. It will also be important to characterize the lasting effects of remapping on behavioral performance, and that remapping measured with IEG activation is not only a transient result of novelty detection (Fyhn, Molden, Hollup, Moser, & Moser, 2002). Further, within-subject designs will serve as a powerful tool to examine changes in cue and spatial representation in the hippocampal memory code, and their relation to behavior across the learning experience. In addition, future studies may examine septal-temporal differences in hippocampal neuron population responses across the learning experience. Some models of multiple memory systems would suggest that the CA1 representation would not maintain a relationship with behavior when cue memory gains control, whereas single-process models such as the HR concept predict there will be a relationship between CA1 population activity for both cue- and place-guided behavior (Lee et al., 2016). Further experiments on this issue will significantly further our understanding of memory organization in the brain.

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