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RESEARCH REPORT

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Hippocampal and prefrontal contributions to memory retrieval: Examination of immediate early gene, NMDA receptor and environmental interactions

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Abstract

Animals can use a range of strategies to recall important locations. These include simple stimulus–response strategies and more complex spatial (place) strategies, which are thought to have distinct neural substrates. The hippocampus—and NMDA receptor activation therein—is considered to be crucial for spatial, but not response strategies. The medial prefrontal cortex has also been implicated in memory retrieval; however, evidence concerning its specific role is equivocal. Both hippocampal and prefrontal regions have been associated with flexible behavioural responding (e.g. when task demands change). Here, we investigated the use of spatial and non-spatial strategies in the Morris water maze and their associated brain areas in rats using immediate early gene (IEG) imaging of Zif268 and c-Fos. Specifically, we charted the involvement of hippocampal and prefrontal subregions during retrieval of spatial and non-spatial memories. Behavioural flexibility was also examined using intact and partial cue configurations during recall. Results indicated that regions of both the hippocampus (area CA3) and prefrontal cortex (anterior cingulate cortex) were preferentially engaged in spatial memory recall compared to response learning. In addition, both spatial and non-spatial memories were dependent on NMDA receptor activation. MK801 impaired recall performance across all groups and reduced IEG activation across hippocampal and prefrontal regions. Finally, IEG results revealed divergent patterns of Zif268 and c-Fos activity and support the suggestion that Zif268 plays a functional role in the recall of long-term memories.

KEYWORDS

glutamate receptors, immediate early gene, Morris water maze, rodents, spatial learning

Abbreviations: ACC, anterior cingulate cortex; CA1, cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus; IEG, immediate early gene; ILC, infralimbic cortex; NE, north-east; NMDA, N-methyl-D-aspartate; NW, north-west; PB, phosphate buffer; PBS, phosphate-buffered saline; PBX, phosphate buffer containing Triton X-100; PLC, prelimbic cortex; SE, south-east; SW, south-west.

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1 | INTRODUCTION

Animals can employ a range of navigational strategies to reach a goal, from simple stimulus–response associations, such as approaching a prominent beacon, to the use of more complex representations based on spatial relationships between available cues in the environment (Farina et al., 2015; Rodrigo, 2002; Whitlock, Sutherland, Witter, Moser, & Moser, 2008). Evidence from existing literature strongly indicates that response and place strategies are supported by distinct neural substrates. Specifically, the hippocampus is considered to be essential for the retrieval of newly acquired place memories, but not for beacon navigation, in the water maze (Broadbent, Squire, & Clark, 2006; de Bruin, Moita, de Brabander, & Joosten, 2001; McDonald & White, 1994; Morris, Garrud, Rawlins, & O'Keefe, 1982; Save & Poucet, 2000; Simon, Stevens, Curtis, & Ramus, 2011; Sutherland & Rodriguez, 1989). With regard to response strategies, the dorsal striatum has been highlighted as an important area (Devan, McDonald, & White, 1999; McDonald & White, 1994; Packard & McGaugh, 1992). In addition, de Bruin and colleagues (de Bruin et al., 2001; de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994) found that rats with medial prefrontal lesions were impaired at navigating to a visible platform in the water maze, but displayed normal retention on a hidden platform version of the task, suggesting that this area is also involved in non-spatial strategies. However, the observed results may equally have reflected a failure to change strategy in keeping with task demands (poor behavioural flexibility), as opposed to a deficit in beacon navigation per se (de Bruin et al., 1994). A study by Jo et al. (2007) investigated regional involvement in behavioural flexibility and strategy switching further. The authors tested rats with lesions to the medial prefrontal cortex, or to hippocampal area CA3, in a hidden platform task under full and partial cue conditions. In the full cue condition, rats were tested with four distal training cues. In the partial cue condition, three of the cues were removed, leaving only one distant cue. Both prefrontal and CA3 lesion groups showed poor retrieval under partial, but not full, cue conditions. In addition, the authors found that navigation with an incomplete cue arrangement elevated the number of immunopositive c-Fos cells in prefrontal and CA3 regions (but not in CA1 or the dentate gyrus). Together with earlier findings, these results are consistent with the suggestion that both the hippocampus and prefrontal cortex are crucial for the flexible use of stored representations, that is under diminished cue conditions (Compton, Griffith, McDaniel, Foster, & Davis, 1997; Jo et al., 2007).

Hippocampal N-methyl-D-aspartic acid (NMDA) receptor activation is critical for spatial learning (Bannerman, Good, Butcher, Ramsay, & Morris, 1995; Lee & Kesner, 2002; Li, Matsumoto, Yamamoto, & Watanabe, 1997; Liang, Hon, Tyan, & Liao, 1994; Martin, Grimwood, & Morris, 2000; Morris, Anderson, Lynch, & Baudry, 1986). However, its exact role is not as clear-cut as previously thought and may depend on both the subregion involved and the nature of the spatial task. For example, Niewoehner et al. (2007) found that NR1 NMDA subunit deletion in the dentate gyrus had no effect on spatial performance in the water maze, while Tsien, Huerta, and Tonegawa (1996) showed deletion of NR1 receptor subunit in CA1 leads to poor acquisition of the water maze task, but no impairment in a non-spatial version of the task. Studies by Nakazawa et al. (2002) and Fellini, Florian, Courtney, and Roullet (2009) found that mutant mice with specific ablation of NMDA receptors in area CA3 successfully acquired and retrieved spatial memories in the water maze task using distal cues, but were unable to navigate when presented with a subset of the original cue configuration. However, Mei, Li, Gu, Cui, and Tsien (2011) illustrated that knockout mice lacking NMDA receptors in CA1 or the entire hippocampus at the time of memory recall were not impaired in a spatial reference memory task under full or partial cue conditions.

As markers of neuronal activity, immediate early gene (IEG) expression has been reported under a variety of spatial conditions (for review, see Barry & Commins, 2011 and also Kubik, Miyashita, & Guzowski, 2007). For example, IEGs, including Zif268 and c-Fos, have been implicated in the consolidation of spatial memories (Barry & Commins, 2017), as well as long-term memory retention, both in a functional capacity and as neuronal markers of regional activation (Barry, Coogan, & Commins, 2016; Guzowski, 2002; Kubik et al., 2007; Lanahan & Worley, 1998). However, research comparing IEG expression patterns associated with spatial and non-spatial memory retrieval is limited. One study carried out by Guzowski, Setlow, Wagner, and McGaugh (2001) examined place and response memory using hidden and visible platform water maze tasks, respectively, measuring hippocampal expression of Zif268, c-Fos and Arc in these groups and in a group of untrained rats. Interestingly, the authors found equivalent increases in hippocampal expression of all IEGs in spatial and non-spatial groups relative to caged controls (Guzowski et al., 2001). While IEG expression within the hippocampus has been examined, patterns of expression outside this region and specifically in the medial prefrontal region are relatively unexplored. Comparisons of IEG expression in hippocampal and prefrontal regions for spatial and non-spatial strategies, as well as behavioural flexibility, are currently limited. Furthermore, how the NMDA receptor and IEGs interact during spatial and non-spatial memories is limited. Building on the work of Farina and Commins (2016), which showed an interaction between NMDA and IEG expression during spatial learning, this paper will focus on retention of spatial and non-spatial

strategies. Specifically, we aim to (a) delineate the involvement of hippocampal and medial prefrontal subregions during the retrieval of spatial and non-spatial memories using IEG imaging; (b) establish the relative importance of NMDA receptors for spatial and non-spatial strategy use; and (c) explore how NMDA receptor blockade during retrieval influences expression of Zif268 and c-Fos in the hippocampus and prefrontal cortex.

2 | MATERIALS AND METHODS

2.1 | Subjects

Forty-two male Wistar rats obtained from Charles River, UK, were used as subjects. Animals were approximately three months old and weighed 250–300 g at the beginning of the experiment. All rats were given a number with a non-toxic marker pen for identification purposes and housed three per cage in plastic-bottomed cages (56 × 38 and 22 cm high; NKP Cages, UK) with a 3 cm layer of wood-chip bedding, paper strip nesting material and cardboard tubes. All rats had access ad libitum to water and food pellets and were maintained under a 12:12-hr light:dark cycle (lights on at 07:00 hr) at a fixed temperature of 21°C. All experimentation was conducted during the light phase. All rats were experimentally naïve and were well-handled for one week prior to the onset of each experiment. Power calculations were performed to determine the appropriate sample size, which was the minimum required to detect within- and between-group differences. Calculations were performed using G*Power (http://www.gpower.hhu.de/). As we were using a repeated-measures ANOVA with within-between interaction across six groups (3 conditions with 2 treatments), with a specified high power of 0.9 and moderate effect size of 0.3, we calculated an overall n of 42 giving seven per group. In addition, previous behavioural investigations in our laboratory using the same water maze apparatus and training protocol have used equivalent sample sizes (Harvey, Brant, & Commins, 2009; Kealy et al., 2008).

2.2 | Apparatus

The Morris water maze was employed as the spatial navigation task and has been used previously in our laboratory (Barry & Commins, 2019; Harvey et al., 2009). The maze consisted of a black, circular fibreglass pool (170 cm diameter, 35 cm deep) resting 70 cm above floor level on a metal support frame. The pool was filled with opaque water to a depth of 20 cm and maintained at 21 ± 1°C. A black concrete escape platform (13 cm diameter, 13.5 cm width) was placed in the centre of the north-east (NE) quadrant of the pool (25 cm from the edge of the pool wall) for all training trials. The platform rested 2 cm below the water surface, ensuring that rats could not see it when navigating in the maze. The maze was surrounded by a black curtain suspended from ceiling to floor at a distance of 60 cm from the pool wall which provided a uniform background and prevented access to extra-maze cues.

Visual, distal cues located in fixed positions around the maze were used to guide the rats to the platform. Cues were fluorescent, inside-frosted, low-energy Philips glass light bulbs, which were suspended from the ceiling inside the curtain. Rats in the spatial groups were trained with two cues of equal brightness: two 25-watt light bulbs. One cue was located in the NE position, distance of 127 cm from platform and height angle of 42°. The second cue was located in the north-west (NW) position, distance of 162 cm and height angle of 25°. Rats in the non-spatial (beacon) groups were trained with a single beacon (25-watt light suspended at 60 cm directly above the platform)—see Figure 1a. These cues were the only light source available. To minimise distraction for the animals (e.g. noise), all trials were observed by the experimenter in an adjacent testing room via a video camera positioned directly above the centre of the maze. Behavioural data of the animals' movements were recorded using EthoVision© tracking system (Noldus Information Technologies, Wageningen, Netherlands).

2.3 | Water maze procedure

2.3.1 | Acquisition

Animals were initially divided into two groups: a spatial group ($n = 28$) and a beacon group ($n = 14$). Animals in the spatial group were trained to find the fixed, hidden platform (in middle of NE quadrant) using two distal cues (described above). The beacon group was also trained to find the platform (in NE quadrant), but with a light suspended directly above the platform. All animals were trained in the maze for ten days and were given 4 × 60 s trials per day. The starting position was rotated across trials, as per Rice, Wallace, and Hamilton (2015). For each trial, rats were placed into the pool near to and facing the pool wall from one of four pseudo-randomised directional starting positions (north, south, east or west). Time taken to reach the platform was recorded. Rats were allowed a maximum of sixty seconds to find the platform. If they failed to locate the platform within this time, rats were guided there by the experimenter. Once on the platform, rats remained there for 15 s after which they were removed from the maze and placed into an open-topped container for an inter-trial interval of 10 s. Rats were placed back into the pool from a different starting position for the

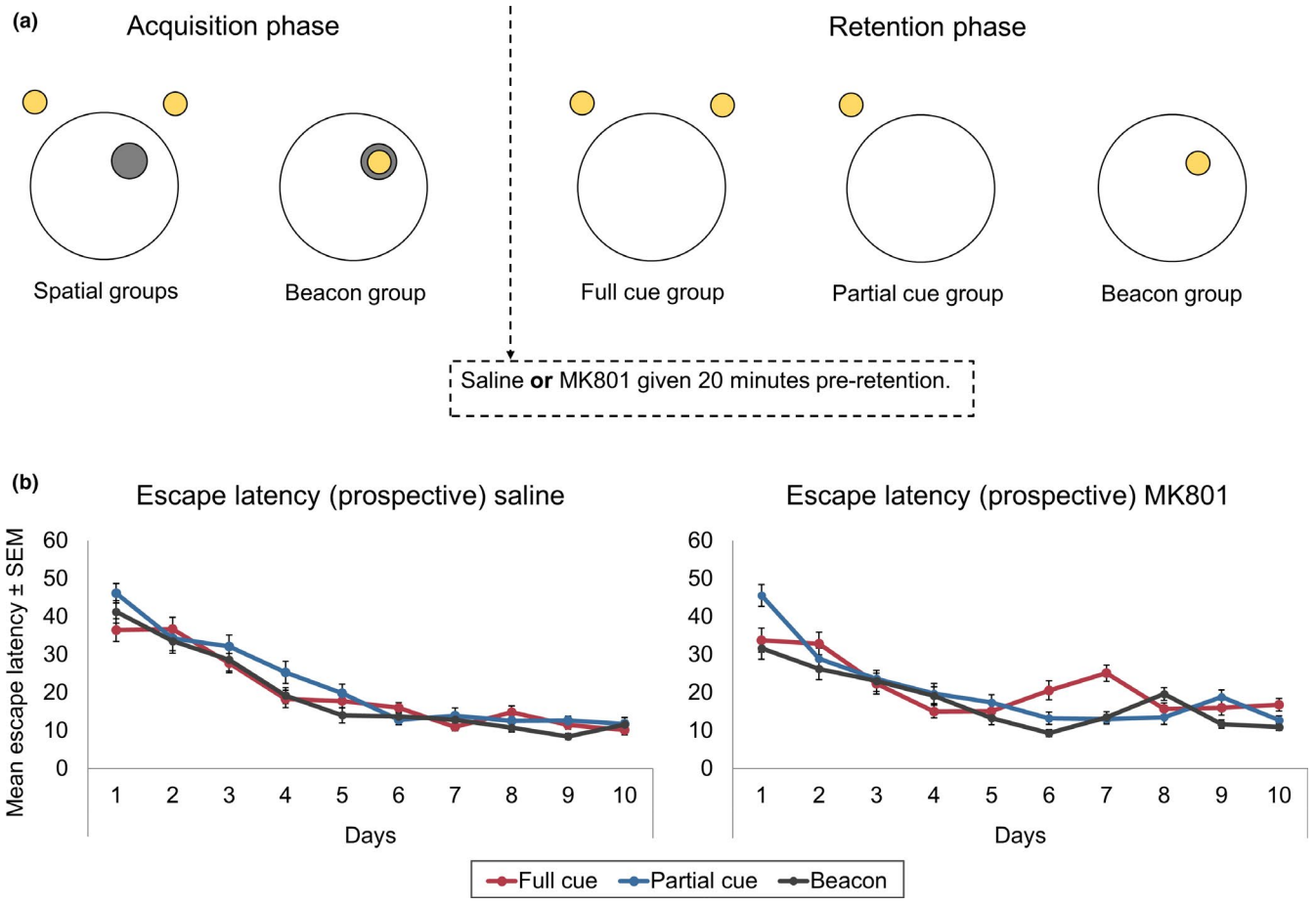

FIGURE 1 (a) Experimental set-up showing how the various groups were trained and subsequently tested. Location of cues is represented by yellow circles outside the large circular arena. The location of the platform (during acquisition) is represented by the small open circle located in the north-east quadrant of the arena. (b) Mean escape latencies (±*SEM*) comparing animals that will be treated with saline or MK801 post-acquisition. No group differences were noted ensuring equivalence in learning [Colour figure can be viewed at wileyonlinelibrary.com]

next trial. When all four daily trials had been completed, rats were returned to their home cage.

2.3.2 | Retention

Memory retention was assessed 24 hr after the final day of training (i.e. on day 11) with one probe trial lasting 60 s. Twenty minutes before testing, each group was further divided and administered with an i.p. injection of saline solution (0.1 ml/100g body weight of 0.9% NaCl) or the NMDA blocker MK801 (0.1 mg/kg body weight, see Farina & Commins, 2016). The spatial groups (saline and MK801) were then re-tested under either *full cue* condition with both near (NE) and far (NW) cues, as per acquisition (saline-full cue; MK801-full cue), or *partial cue* condition, with only the far (NW) cue present (saline-partial cue; MK801-partial cue; $n = 7$ per group). The beacon groups (saline or MK801, $n = 7$ per group) were re-tested with the beacon, as per acquisition (see Figure 1a for experimental set-up). A novel start position was used for the retention trial. All rats were placed into the maze near to and facing the wall from the centre of the south-west (SW) quadrant.

2.4 | Tissue preservation

Rats were terminally anaesthetised via i.p. injection with sodium pentobarbital (60 mg/kg, Euthatal) 90 min post-retention (i.e. on day 11). Ninety minutes was chosen as IEGs are maximally expressed at this time point (Zangenehpour & Chaudhuri, 2002; Barry & Commins, 2017; see also Teixera, Pomedli, Maei, Kee, & Frankland, 2006). Rats were then perfused transcardially with 0.9% phosphate-buffered saline (PBS, 250 ml, Ph 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, 300 ml, Ph 7.4). Brains were immediately removed and post-fixed in 4% paraformaldehyde overnight at 4°C before being cryoprotected in 30% sucrose solution. Brains were then frozen on dry ice and cut into 40-μm-thick coronal sections using a freezing stage sledge microtome (Bright Instruments, Huntingdon, UK). Free-floating sections were stored in 0.1

M PB containing 0.01% sodium azide (4°C). Subregions of the hippocampus (CA1, CA3 and dentate gyrus, DG) and medial prefrontal cortex (prelimbic cortex, PLC; anterior cingulate cortex, ACC; infralimbic cortex, ILC) were included in IEG imaging analyses (four sections per region). Hippocampal sections were taken from Bregma −3.16 to −4.12 and prefrontal sections taken from Bregma +3.14 to +2.76 (Paxinos & Watson, 2007).

2.5 | Immunohistochemistry

Standard immunohistochemical staining methods were followed (Coogan & Piggins, 2003). Sections were washed twice in 0.1 M PB (ten minutes each), followed by a ten-minute wash in 0.1 M PB containing 0.2% Triton X-100 (PBX). Sections were then washed in 0.1 M PB with 1.5% hydrogen peroxide for 20 min. Two more ten-minute washes in 0.1 M PB and one in PBX followed. Sections were then blocked in 5% normal goat serum (NGS) in 0.1 M PBX for sixty minutes at room temperature and incubated for 24 hr in a primary antibody solution (2% NGS in 0.1 M PBX). Zif268 and c-Fos were labelled using the following antibodies: Zif268/Egr-1, rabbit polyclonal antibody (dilution 1:3,000; Santa Cruz Biotechnology); c-Fos, rabbit polyclonal antibody (dilution 1:2,000; Santa Cruz Biotechnology).

Post-incubation, sections were given two washes in 0.1 M PB and one in PBX. Sections were then incubated with biotinylated secondary antibody (goat anti-rabbit, Jackson Laboratories, dilution 1:400) for seventy minutes. Two more washes in 0.1 M PB and one in 0.1 M PBX followed, after which sections were incubated with avidin–biotin–peroxidase complex (0.4%; Vector Laboratories) for ninety minutes in complete darkness at room temperature. Sections were again washed twice more in PB and once in 0.1 M sodium acetate (Ph 6). The reaction product was visualised using the nickel–DAB technique with glucose oxidase (Sigma, Poole, UK) as the catalyst. The length of reaction time was standardised for all sections to ensure comparable staining intensity across sections. Finally, sections were mounted onto gelatin-coated slides, dehydrated, cleared in Histo-Clear (National Diagnostics, Hull, UK) and coverslipped using Eukitt (Sigma, Poole, UK).

2.6 | Data analysis

Acquisition of the water maze task was measured by the mean time it took animals to find the hidden platform – escape latency (seconds). Mean trial values for each rat were averaged to produce group means. Retention was examined as percentage time spent in the platform areas for each group. The platform area was defined as a circular area around where the platform was previously located (NE), comprising 7% of the total searchable area of the maze, and compared to three other equivalent areas of the maze (NW, SE and SW).

Images of the hippocampal (CA1, CA3 and DG) and medial prefrontal cortex (PLC, ACC and ILC) regions were taken using an Olympus digital camera (CAMEDIA C-2020-Z) mounted on an Olympus BX-50 microscope. To capture the maximum number of cells possible, all images were taken using a 4× magnification. Numbers of Zif268 and c-Fos immunopositive were counted using ImageJ software (National Institute of Health, USA), and group means were obtained. In order for the software to distinguish active cells from inactive background tissue, a number of detection thresholds were used. These included brightness intensity (set between 70 and 100) and particle size (20 to 200 pixel range). Counts from each animal (from four sections) were averaged to produce a mean cell count per region per animal.

2.7 | Statistical analysis

Statistical analyses were carried out using SPSS version 22 (IBM, New York, USA). Group differences in escape latencies in each training condition were analysed using mixed factorial ANOVAs, with group as the between-groups factor (full cue, partial cue and beacon group) and training day as the within-groups factor (days 1 to 10). Time spent in platform areas for treatment groups (saline and MK801) and across conditions (full cue, partial cue and beacon) was also analysed using mixed factorial ANOVAs. Where Mauchly's test of sphericity was violated, Greenhouse–Geisser corrections were applied. Otherwise, Wilk's lambda is reported. Tukey and Bonferroni *post hoc* tests were included in these analyses where appropriate. Zif268 and c-Fos expression in the different regions were examined with a number of mixed factorial ANOVAs. Bonferroni-corrected *t* tests were used to compare across brain regions, and Tukey *post hoc* comparisons were used to compare groups. Alpha level of 0.05 was used as the significance criterion for all statistical analyses. Raincloud plots were created using the website https://gabrifc.shinyapps.io/raincloudplots/.

2.8 | Ethics

Guidelines for the maintenance and experimentation of animals conformed to the Department of Health and Children (Ireland) guidelines under statutory instrument (S.I.) No. 543 of 2012 and the European Directive 2010/63/EU. The Maynooth University Ethics Committee also approved all experimental work.

3 | RESULTS

3.1 | Behavioural results

3.1.1 | Acquisition

All spatial groups were trained under the same experimental conditions. Drug treatments (saline or MK801) and environmental manipulation (full or partial cue) were administered *post-acquisition*. However, because we wanted to ensure that all groups learned the task equally, we separated rats into their prospective groups for acquisition analysis.

First, we conducted a Treatment (saline; MK801) × Day (1–10) mixed factorial ANOVA to examine whether the prospective drug treatment groups learned equivalently. We found no effect of Treatment ($F_{1,40} = 0.233$, $p = .632$, partial $\eta^2 = 0.006$). The main effect of Day was significant ($F_{9,360} = 62.29$, $p = .001$, partial $\eta^2 = 0.61$; Figure 1b). Next, we conducted a Group (full cue; partial cue; beacon) × Day (1–10) mixed factorial ANOVA to compare prospective environmental manipulations for the *saline-treated* groups. We found a significant main effect of Day ($F_{9,162} = 39.29$, $p = .001$, partial $\eta^2 = 0.69$), but no Group ($F_{1,18} = 1.31$, $p = .30$, partial $\eta^2 = 0.13$) or Day × Group ($F_{18,162} = 0.60$, $p = .90$, partial $\eta^2 = 0.62$) interaction effects. Bonferroni *post hoc* tests indicated that rats were significantly faster at escaping the pool on day 10 than on day 1 ($p = .001$). Similarly, we conducted a Group × Day mixed factorial ANOVA to compare prospective environmental manipulations for the *MK801-treated* groups. This analysis also yielded a main effect of Day ($F_{9,162} = 27.27$, $p = .001$, partial $\eta^2 = 0.60$) and a Day × Group interaction effect ($F_{18,162} = 2.68$, $p = .01$, partial $\eta^2 = 0.23$). Escape latency on day 10 was significantly shorter than on day 1 ($p = .001$). The main effect of Group was not significant ($F_{1,18} = 1.22$, $p = .32$, partial $\eta^2 = 0.12$). To follow up the significant interaction effect, we conducted separate repeated-measures ANOVAs for each group to assess learning across days. Significant main effects of Day were found for all groups (full cue: $F_{9,243} = 6.48$, $p = .001$, partial $\eta^2 = 0.19$; partial cue: $F_{9,243} = 15.20$, $p = .001$, partial $\eta^2 = 0.36$; beacon: $F_{9,243} = 8.71$, $p = .001$, partial $\eta^2 = 0.24$). Importantly, all groups were significantly faster at escaping the pool on day 10 compared to day 1 (all Ps < 0.02). These results suggested that all groups learned the task equally well.

3.2 | Retention

Memory retention was assessed by allowing all animals to swim in a platform-less pool, 24 hr after the final day of training (i.e. day 11), for 60 s. The full cue groups (saline and MK801) were re-tested with two cues present, as per acquisition, but the partial cue groups (saline and MK801) were re-tested with just one of the distal cues present (i.e. the NW cue). The beacon groups (saline and MK801) were re-tested with the cue suspended above the platform, as per acquisition.

Initially, we examined the effect of MK801 on spatial recall overall, irrespective of the environmental manipulation. A 2 × 4 mixed factorial ANOVA demonstrated an overall effect of Treatment ($F_{1,40} = 41.43$, $p = .001$; partial $\eta^2 = 0.51$), with MK801 significantly impairing recall compared to saline (Figure 2a). There was also a significant effect of Area ($F_{3,\ 120} = 25.71$, $p = .001$; partial $\eta^2 = 0.39$) and a Treatment × Area interaction effect ($F_{3,\ 120} = 8.6221$, $p = .001$. partial $\eta^2 = 0.177$). *Post hoc* tests showed that the saline-treated animals spent significantly more time searching in the target NE area compared to the MK801-treated animals.

We then analysed the time spent in the platform areas for the *saline-treated groups* (Figure 2b-c). A3 (full, partial and beacon groups) × 4 (platform areas) mixed factorial ANOVA was conducted. A significant main effect of Area ($F_{3,54} = 29.48$, $p = .001$, partial $\eta^2 = 0.62$) and Group × Area interaction ($F_{6,54} = 3.50$, $p = .02$, partial $\eta^2 = 0.28$) effect was found. No Group effect was noted ($F_{1,18} = 2.15$, $p = .15$, partial $\eta^2 = 0.19$). Bonferroni *post hoc* tests revealed that the groups spent longer in the NE area than in other areas. A one-way ANOVA was done comparing the time spent by each of the three groups in the NE area; no main effect was found ($F_{2,20} = 3.25$, $p = .06$). Examining each group separately showed an overall significant effect of area for the full cue ($F_{3,18} = 26.07$, $p = .001$, partial $\eta^2 = 0.81$) and beacon groups ($F_{3,18} = 8.31$ $p = .03$, partial $\eta^2 = 0.58$) with both showing a preference for the NE area compared to other areas. No main effect was found for the partial cue group ($F_{3,18} = 2.71$, $p = .08$, partial $\eta^2 = 0.31$), suggesting that although this group searched generally in the NE quadrant, they were less accurate compared to the other groups (Figure 2b-c). Note that this group also spent more time in the starting position (SW, $p < .05$).

Analyses of time spent in platform areas for *MK801-treated groups* (Figure 2c) were then done using a 3 × 4 mixed factorial ANOVA. Main effects of Area ($F_{3,54} = 3.99$, $p = .04$, partial $\eta^2 = 0.18$) and Group ($F_{1,18} = 4.38$, $p = .03$, partial $\eta^2 = 0.33$) were significant. The Area × Group interaction effect was not significant ($F_{6,54} = 2.01$, $p = .13$, partial $\eta^2 = 0.18$). Bonferroni *post hoc* tests failed to indicate any differences across areas. However, Tukey *post hoc* comparisons did highlight a significant difference between the partial cue and beacon groups ($p = .04$), with the partial cue group performing significantly worse. Repeated-measures ANOVAs were then carried out to investigate differences in time spent across areas for each group separately; however, no main effects of area were found; full cue group, $F_{3,18} = 4.99$, $p = .06$, partial $\eta^2 = 0.45$, partial cue group, $F_{3,18} = 2.71$, $p = .22$, partial $\eta^2 = 0.22$, beacon group, $F_{3,18} = 0.51$, $p = .68$, partial

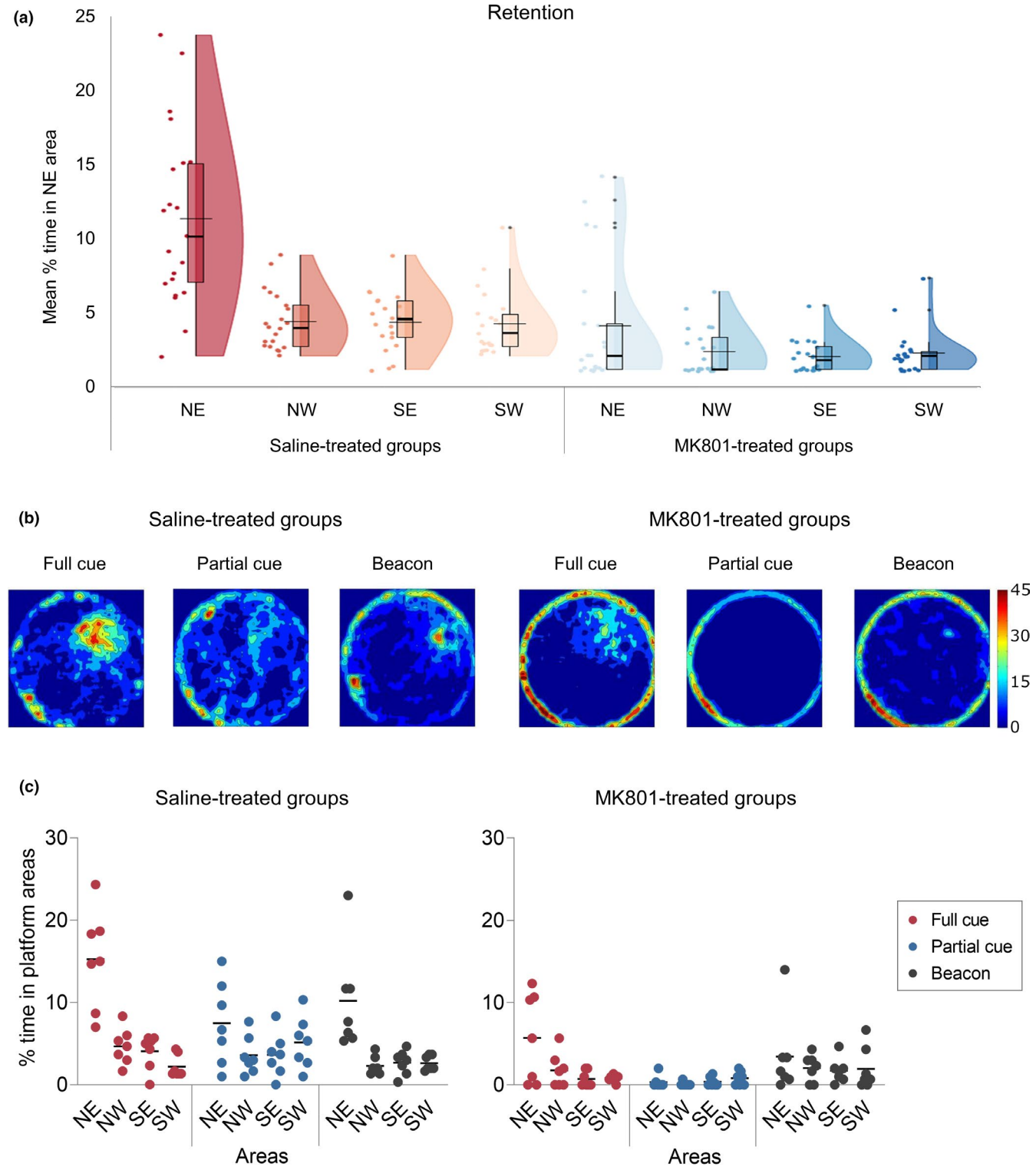

FIGURE 2 (a) Boxplot, individual scores and distribution of time spent by the saline-treated (red) and MK801-treated groups (blue) in each of the platform areas (including the target NE area) during retention. Dark horizontal line in boxplot = median; long light horizontal line = mean. (b): Heat maps showing search distributions for saline- and MK801-treated animals in full cue, partial cue and beacon groups. (c) Percentage time spent in platform areas by *saline-treated* groups and *MK801-treated* groups during the retention trial. Black line indicates group mean [Colour figure can be viewed at wileyonlinelibrary.com]

$\eta^2 = 0.08$. These results suggest that MK801 had a profound effect on the ability of any group to recall the platform's location, particularly the partial cue group.

In order to check for potential sensorimotor effects caused by MK801, we examined mean distance travelled and velocity for the saline- and MK801-treated groups. No

significant group differences we found for distance travelled (saline = 1725.8 ± 1.2 cm; MK801 = 1655.6 ± 1.0 cm; $t = 0.686$, $df = 40$, $p = .496$) or velocity (saline = 29.4 ± 1.2 cm; MK801 = 28.67 ± 1 cm; $t = 0.435$, $df = 40$, $p = .666$). These results indicate that treatment with MK801 did not lead to sensorimotor impairments.

3.3 | IEG results

3.3.1 | Zif268

Similar to the behavioural data, we initially examined the effect of MK801 on IEG expression in the hippocampus, irrespective of the environmental manipulation. Figure 3a demonstrates that the number of Zif268-positive cells was significantly reduced in the hippocampal regions ($t_{123} = 14.217$, $p = .001$) in MK801-treated animals (2.3 ± 0.3) compared to saline-treated animals (130.2 ± 9, Figure 3a). We then compared the mean number of Zif268-positive cells across each hippocampal region (CA1, CA3 and DG) for each environmental condition (full cue, partial cue and beacon) for those animals treated with *saline* (Figure 3b). A 3 × 3 mixed factorial ANOVA was conducted. A significant main effect of Area ($F_{2,36} = 19.23$, $p = .001$, partial $\eta^2 = 0.52$) was found, with Bonferroni *post hoc* tests showing significantly more Zif268 expression in area CA1 compared to the other hippocampal regions. No Group × Area interaction

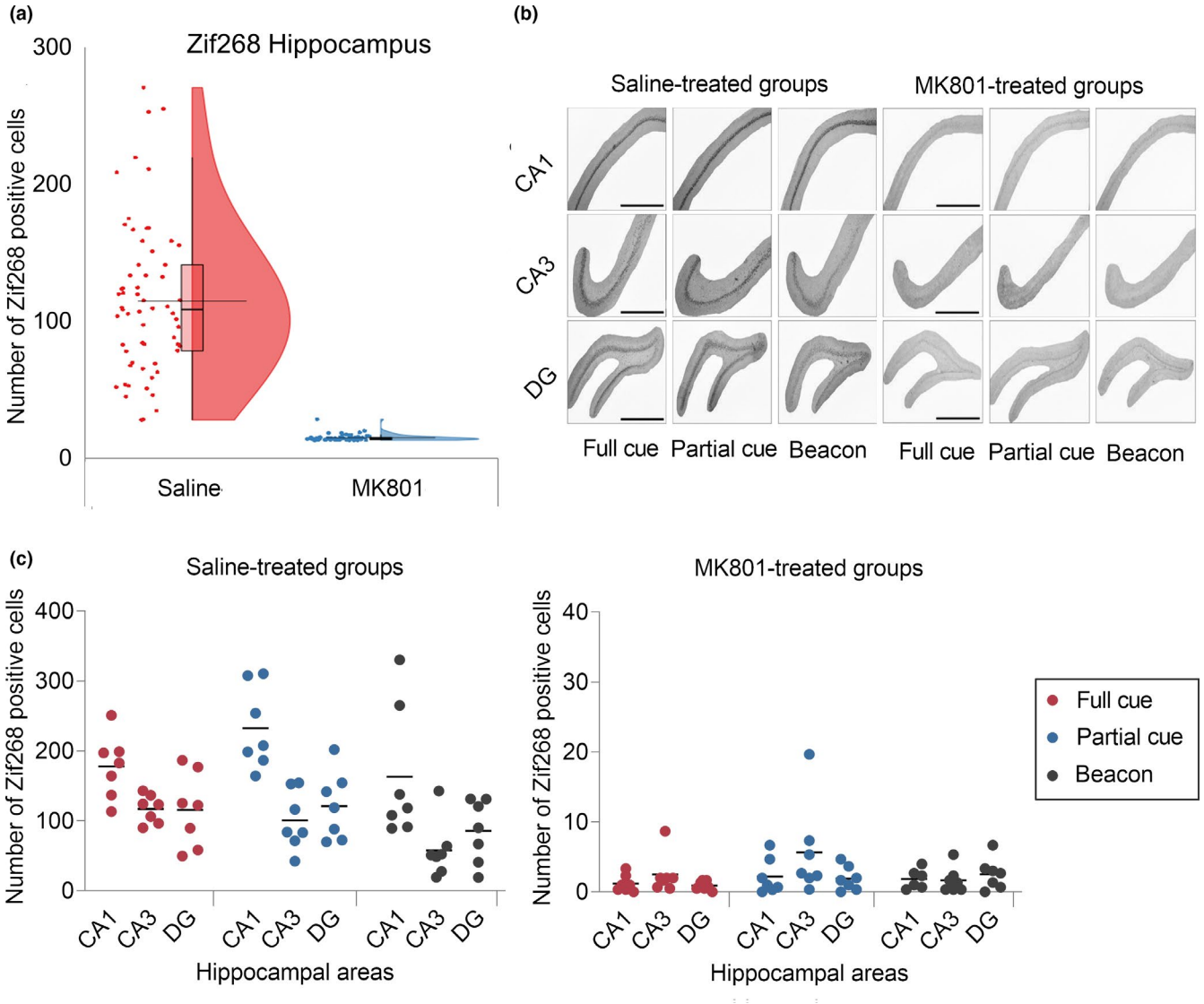

FIGURE 3 (a) Number of Zif268-positive cells in hippocampus for saline-treated (red) and MK801-treated (blue) animals, shown individually (dots), with boxplots (dark horizontal line = median; long horizontal line = mean) and distribution. (b): Representative images of Zif268 expression for each area and environmental condition. Scale bar = 1 mm. (c): Scatterplots showing individual raw Zif268 counts and mean (horizontal line) for each environmental condition (full cue, partial cue and beacon) for hippocampal areas CA1, CA3 and DG (dentate gyrus) for saline- and MK801-treated animals. Note: the different y-axis ranges [Colour figure can be viewed at wileyonlinelibrary.com]

($F_{4,36} = 0.815$, $p = .524$, partial $\eta^2 = 0.083$) effect was found. However, an overall significant Group effect was noted ($F_{2,18} = 5.786$, $p = .011$, partial $\eta^2 = 0.39$), with Tukey *post hoc* tests showing significantly less Zif268 expressed in the beacon group compared to the partial cue group overall. In area CA3, Zif268 expression was also significantly less in the beacon group compared to full cue group ($F_{2,18} = 5.139$, $p = .017$, see Figure 3b). Following this, we compared the mean number of Zif268-positive cells across each hippocampal region and environmental conditions for those animals treated with *MK801* (Figure 3c). A 3 × 3 mixed factorial ANOVA was again conducted for the MK801-treated but no Area ($F_{2,34} = 2.29$, $p = .117$, partial $\eta^2 = 0.12$), Group × Area ($F_{4,34} = 2.48$, $p = .062$, partial $\eta^2 = 0.226$) or Group effect ($F_{2,17} = 1.23$, $p = .317$, partial $\eta^2 = 0.126$) was found.

Examination of the effect of MK801 on IEG expression in the prefrontal cortex, irrespective of the environmental manipulation, demonstrated that the number of Zif268-positive cells was significantly reduced ($t_{123} = 8.488$, $p = .001$) in the MK801-treated animals (17.9 ± 3.0) compared to those that were given saline (386.9 ± 43, Figure 4a). We then compared the mean number of Zif268-positive cells across each prefrontal region (PLC, ACC and ILC) for each environmental condition (full cue, partial cue and beacon) for those animals treated with *saline* (Figure 4b). A 3 × 3 mixed factorial ANOVA was conducted. A significant main effect of Area ($F_{2,36} = 18.83$, $p = .001$, partial $\eta^2 = 0.51$) was found, with Bonferroni *post hoc* tests showing significantly less Zif268 expression in area ILC compared to the other prefrontal regions. An overall Group × Area interaction ($F_{4,36} = 5.163$,

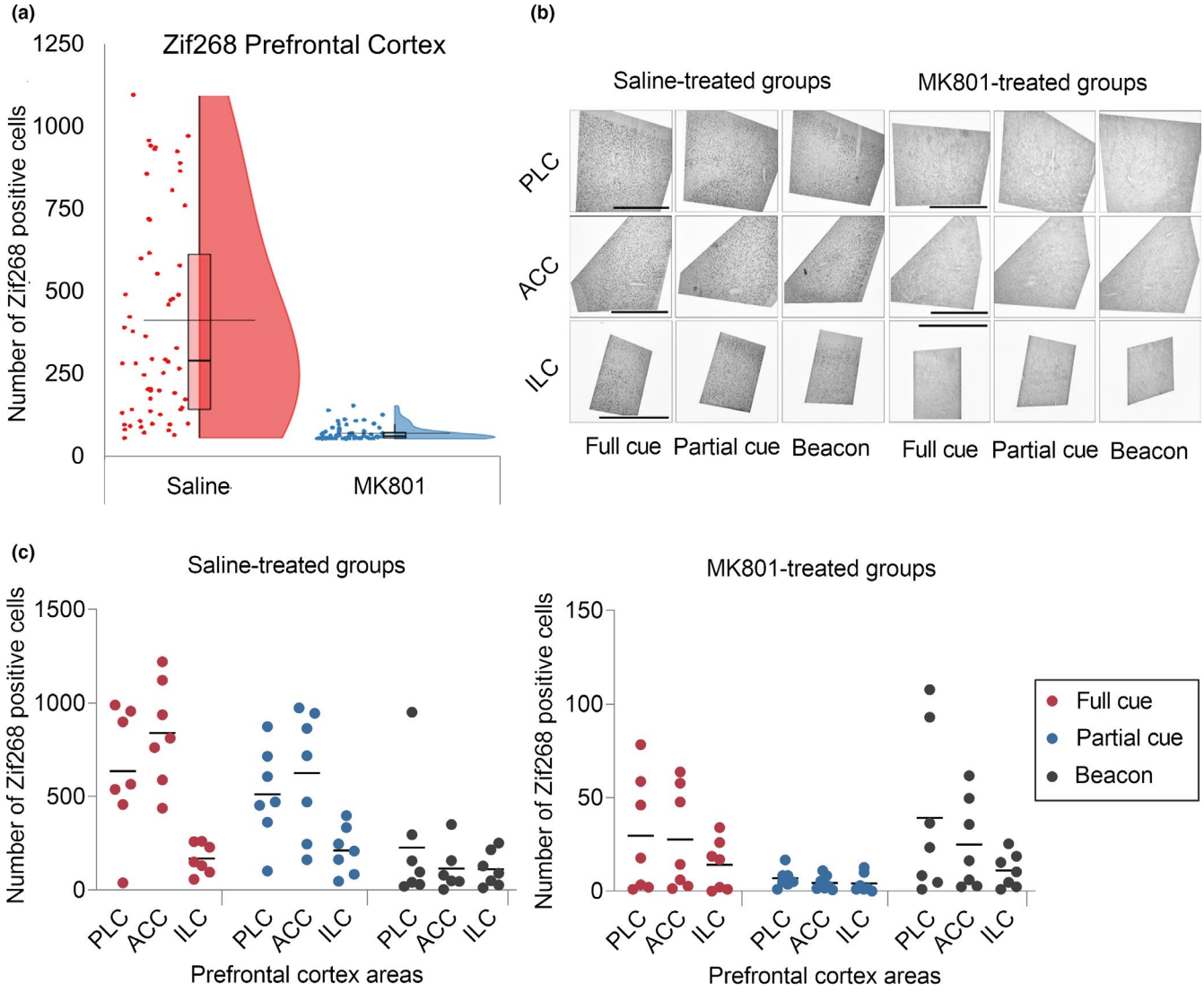

FIGURE 4 (a) Number of Zif268-positive cells in prefrontal cortex for saline-treated (red) and MK801-treated (blue) animals, shown individually (dots), with boxplots (dark horizontal line = median; long horizontal line = mean) and distribution. (b): Representative images of Zif268 expression for each area and environmental condition. Scale bar = 1 mm. (c): Scatterplots showing individual raw Zif268 counts and mean (horizontal line) for each environmental condition (full cue, partial cue and beacon) for prefrontal areas PLC (prelimbic cortex), ACC (anterior cingulate cortex) and ILC (infralimbic cortex) for saline- and MK801-treated animals. Note: the different *y*-axis ranges [Colour figure can be viewed at wileyonlinelibrary.com]

$p = .002$, partial $\eta^2 = 0.365$) and Group effect were noted ($F_{2,18} = 9.817$, $p = .001$, partial $\eta^2 = 0.522$). Tukey *post hoc* tests showing significantly less Zif268 expressed in the beacon group compared to the other two groups generally. This is observed especially in the ACC ($F_{2,18} = 14.95$, $p = .001$, see Figure 4b). A 3 × 3 mixed factorial ANOVA was then for the *MK801-treated* groups (Figure 4c). An overall significant effect for Area ($F_{2,36} = 5.06$, $p = .012$, partial $\eta^2 = 0.22$) was found, with significantly less Zif268 in ILC compared to the PLC. No Group × Area ($F_{4,36} = 1.26$, $p = .30$, partial $\eta^2 = 0.126$) or Group effect ($F_{2,18} = 2.55$, $p = .106$, partial $\eta^2 = 0.221$) was found.

3.4 | c-Fos

Cell counts for c-Fos expression were very low general throughout the hippocampal region. For this region, no difference was noted ($t_{40} = 1.029$, $p = .310$) between those animals treated with saline (5.15 ± 1.2) or MK801 (3.7 ± 0.67). For the *saline-treated* animals, a significant effect for Area was noted ($F_{2,36} = 5.99$, $p = .006$, partial $\eta^2 = 0.247$) with area CA3 expressing more c-Fos than area CA1. However, no Group (full, partial and beacon), $F_{2,18} = 1.815$, $p = .191$, partial $\eta^2 = 0.168$, or Area × Group interaction effect ($F_{4,36} = 2.058$, $p = .107$, partial $\eta^2 = 0.186$) was found (see Figure S1). For the *MK801-treated* animals, a significant effect for Area was again noted ($F_{2,34} = 3.752$, $p = .034$, partial $\eta^2 = 0.181$) but Bonferroni-corrected t tests could not identify where that difference occurred. Again, no Group ($F_{2,17} = 0.658$, $p = .531$, partial $\eta^2 = 0.072$) or Area × Group interaction effect ($F_{4,34} = 0.352$, $p = .841$, partial $\eta^2 = 0.04$) was found (see Figure S1).

We did similar analysis for the prefrontal region; no difference was noted ($t_{40} = 0.525$, $p = .602$) between those animals treated with saline (15.5 ± 4.4) or MK801 (18.6 ± 3.7). For the *saline-treated* animals, again no significant effect for Area ($F_{2,36} = 2.686$, $p = .082$, partial $\eta^2 = 0.13$), Group (full, partial and beacon) ($F_{2,18} = 0.894$, $p = .426$, partial $\eta^2 = 0.09$) or Area × Group interaction effect ($F_{4,36} = 0.886$, $p = .482$, partial $\eta^2 = 0.09$) was found (see Figure S2). For the *MK801-treated* animals, no significant effect for Area ($F_{2,36} = 1.503$, $p = .236$, partial $\eta^2 = 0.07$), Group (full, partial and beacon) ($F_{2,18} = 0.271$, $p = .765$, partial $\eta^2 = 0.03$) or Area × Group interaction effect ($F_{4,36} = 0.072$, $p = .99$, partial $\eta^2 = 0.008$) was found (see Figure S2).

4 | DISCUSSION

The aim of this study was to identify specific hippocampal and prefrontal subregions implicated in the retrieval of spatial and non-spatial memories. Previous research has shown that the hippocampus is crucial for spatial memory recall (Morris et al., 1982; Save & Poucet, 2000) and flexible responding (Jo et al., 2007), but not for non-spatial memory (Packard & McGaugh, 1992). Therefore, we expected to see overall increases in hippocampal IEG expression for both spatial groups (full cue and partial cue) relative to the beacon group. As anticipated, we did see lower Zif268 expression in the hippocampal region for the beacon group, but this was confined to area CA3. This finding is consistent with CA3's suggested role in both spatial recall and flexible responding (Jo et al., 2007). Interestingly, no differences were found between spatial and non-spatial groups in either area CA1 or DG. Although lesion studies have established that non-spatial memory recall can be accomplished by animals with hippocampal lesions, suggesting that this form of memory is not hippocampal dependent (McDonald & White, 1994), beacon responding may still engage specific regions of the hippocampus (e.g. CA1 and DG but not CA3) if intact (Jenkins, Amin, Harold, Pearce, & Aggleton, 2003; Simon et al., 2011). The medial prefrontal cortex has also been implicated in behavioural flexibility, particularly with regard to strategy switching (de Bruin et al., 1994)—although the precise roles of specific subregions remain unclear (Kubik et al., 2007). Accordingly, we predicted that the highest prefrontal IEG expression would also be found in the partial cue group (i.e. animals that were tested with a subset of the original cue configuration). Contrary to our hypothesis, we observed a difference between the spatial groups and the beacon group, particularly in the ACC. This would suggest that the prefrontal cortex may be involved in flexible behaviour as well as the recall of complex, spatial information. Similar to the hippocampus, our findings suggest that the prefrontal cortex plays a limited role in response learning.

We also explored the effects of NMDA receptor blockade on spatial and non-spatial memory retrieval. NMDA receptor activation has been shown to be heavily involved in spatial learning (Farina & Commins, 2016; Morris et al., 1986). Importantly, recent evidence suggests that NMDA blockade is the primary cause of spatial deficits (Morris, Steele, Bell, & Martin, 2013), as opposed to being secondary to sensorimotor deficits caused by NMDA antagonism (previously suggested by Cain, Saucier, Hall, Hargreaves, & Boon, 1996). While a number of studies have reported that MK801 impairs spatial acquisition and not recall (e.g. McLamb, Williams, Nanry, Wilson, & Tilson, 1990), particularly in animals that are well-trained (Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992), we found that MK801 impaired recall of the platform location here. This is consistent with findings from van der Staay, Rutten, Erb, and Blokland (2011) who reported a similar recall impairment in rats treated with the same dose of MK801 (i.e. 0.1 ml per kg). Notably, however, examination of Figure 2a shows that all animals were not impaired and some did spend time searching in the target area.

Additionally, although no differences were noted between groups with regard to time spent in platform areas, memory recall under partial cue conditions appeared to be the most affected by MK801. These results suggest that blockade of NMDA receptors negatively affected the flexible use of stored spatial representations. This finding fits well with previous research (Fellini et al., 2009; Nakazawa et al., 2002), which has shown that deletion or inactivation of NMDA receptors in CA3 alone can impair retention under partial cue conditions. Therefore, it is not surprising that blockade of NMDA throughout the brain resulted in comparable effects here. In addition, Bannerman et al. (2012) indicated that NMDA receptors in area CA1 and DG may play a particular role in using spatial information to select between alternative responses. Although the authors in this study used identical beacons, we trained animals with identical distal cues, each providing spatial information; thus, it is possible that rats in our partial cue group were unable to use the spatial information provided by remaining cue in order to select an appropriate strategy. Our results also indicated that NMDA receptors appear crucial for response learning, thus highlighting the significant role played by NMDA receptors in multiple types of navigational strategies (Vorhees & Williams, 2014). As well as impairing recall, MK801 significantly impacted the expression of Zif268 throughout all regions of both the hippocampus and medial prefrontal cortex. A decrease in Zif268 expression with MK801 treatment has also been observed in the amygdala using tasks examining instrumental memories (Piva et al., 2018).

In contrast to Zif268, no group differences in c-Fos expression were found in any subregion after training (see Farina & Commins, 2016). This result is in line with those of Guzowski et al. (2001), who also failed to find differences in hippocampal c-Fos expression between spatial and non-spatial groups. However, they are inconsistent results from Jo et al. (2007), who reported higher c-Fos expression in CA3 and the prefrontal cortex in a partial cue condition. These divergent findings can most likely be accounted for by variations in the experimental procedures used, such as retention intervals (ranging between 30 min and 24 hr), the number of cues present during initial training (between two and four cues) or indeed the low levels of expression that we observed. On the whole, it appears that although c-Fos is activated during long-term memory retrieval (Barry et al., 2016; Fleischmann et al., 2003), its expression may not be as sensitive to differences underlying spatial and non-spatial strategies in the context of this study. As numbers of c-Fos expressing cells were generally low, even in the saline-treated animals, our results are difficult to interpret. However, we tentatively suggest that Zif268 may be a more useful indicator of regional activation during memory retrieval compared to c-Fos and support the suggestion that Zif268 plays an important functional role in the recall of long-term memories (Jones et al., 2001). More generally, these divergent patterns of IEG activation (see also Shires & Aggleton, 2008) highlight the importance of using multiple markers of neural activity in order to obtain a more informed understanding of regional activation.

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AUTHOR CONTRIBUTIONS

FRF ran the experiments, analysed data, and wrote and read the manuscript. SC formulated the overall concept, and wrote and read the paper.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Supporting data and materials can be accessed on the Open Science Framework:osf.io/f3gvh.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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