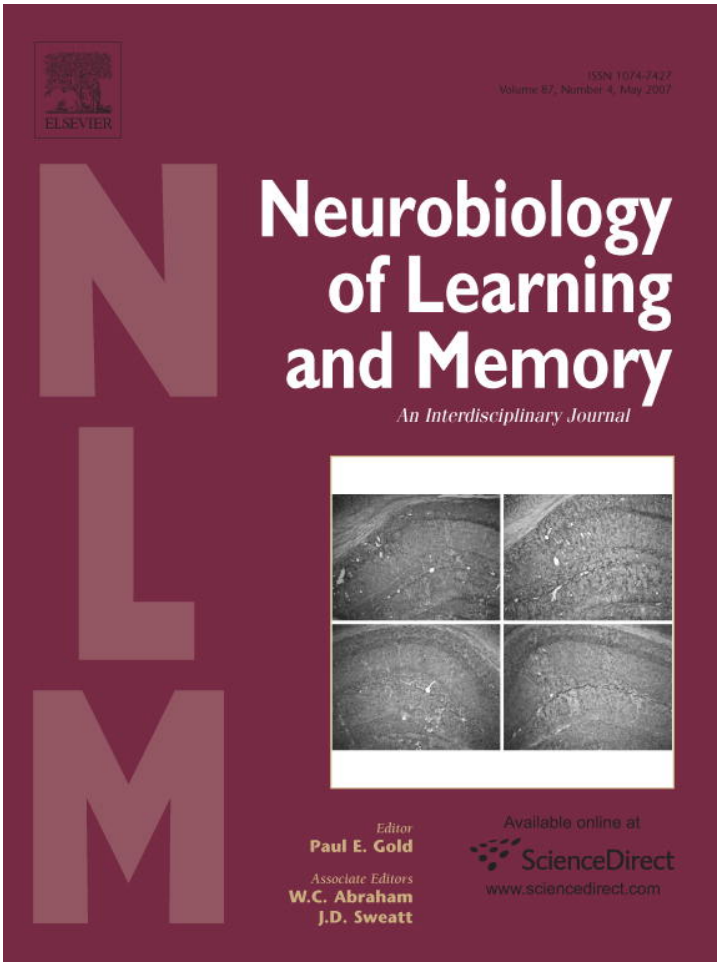

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Immediate early gene activation in hippocampus and dorsal striatum: Effects of explicit place and response training

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Abstract

Evidence from lesion, electrophysiological, and neuroimaging studies support the hypothesis that the hippocampus and dorsal striatum process afferent inputs in such a way that each structure regulates expression of different behaviors in learning and memory. The present study sought to determine whether rats explicitly trained to perform one of two different learning strategies, spatial or response, would display disparate immediate early gene activation in hippocampus and striatum. c-Fos and Zif268 immunoreactivity (IR) was measured in both hippocampus and striatum 30 or 90 min following criterial performance on a standard plus-maze task (place learners) or a modified T-maze task (response learners). Place and response learning differentially affected c-Fos-IR in striatum but not hippocampus. Specifically, explicit response learning induced greater c-Fos-IR activation in two subregions of the dorsal striatum. This increased c-Fos-IR was dependent upon the number of trials performed prior to reaching behavioral criterion and accuracy of performance during post-testing probe trials. Quantification of Zif268-IR in both hippocampus and striatum failed to distinguish between place and response learners. The changes in c-Fos-IR occurred 30 min, but not 90 min, post-testing. The synthesis of c-Fos early in testing could reflect the recruitment of key structures in learning. Consequently, animals that were able to learn the response task efficiently displayed greater amounts of c-Fos-IR in dorsal striatum.

Keywords: c-Fos; Zif268; Place learning; Response learning; Hippocampus; Dorsal striatum; T-maze

1. Introduction

Damage to individual brain regions can cause selective behavioral impairments that are often attributed to functional specializations as independent memory systems. Hippocampus (HPC) and dorsal striatum (DS) are examples of two brain structures that have been categorized based on their proposed involvement in distinct memory systems. Animals with HPC damage are typically impaired on tasks requiring effective use of spatial context information. For instance, HPC lesions impair an animal's ability to utilize spatial landmarks to associate a location with either food reward, as in the plus-maze task, or safety, as in the Morris water maze (McDonald & White, 1993; Packard & McGaugh, 1996). In contrast, the association between discrete stimuli, irrespective of any relationship with spatial cues, and explicit behavioral responses learned through reinforcement outcomes in similar testing conditions appear to rely more on an intact DS (Devan, McDonald, & White, 1999; Featherstone & McDonald, 2005).

In certain instances, there appears to be competition between HPC and DS to regulate behavioral output (reviewed in Mizumori, Yeshenko, Gill, & Davis, 2004). Inactivation, or lesion, of HPC can cause simultaneous impairment of spatial learning and facilitation of acquisition of a response task (Chang & Gold, 2003). In conflict with the proposal that DS mediates only stimulus–response behaviors, lesions of a specific subregion, dorsomedial (DM) of DS can interfere with spatial and response learning. (Devan, Goad, & Petri, 1996; Whishaw, Mittleman, Bunch, & Dunnett, 1987). This would suggest that the functional division of HPC and DS into completely separate memory systems may be too restrictive.

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At even greater odds with the multiple memory systems theory, single-unit recordings in HPC and DS have illustrated remarkable similarities in terms of spatial representation. Both regions contain neurons that exhibit spatially selective neural activity (Gill & Mizumori, 2006; Mizumori, Cooper, Leutgeb, & Pratt, 2000; Ragozzino, Leutgeb, & Mizumori, 2001; Yeshenko, Guazzelli, & Mizumori, 2004). Location-specific firing in both HPC and STR is sensitive to alterations in the visual testing environment independent of whether animals are performing a place or response task (Yeshenko et al., 2004). Despite the similarities in HPC and DS response to contextual changes, differences in how these areas respond to dopaminergic manipulations suggest that each region is differentially regulated by dopamine (Gill & Mizumori, 2006).

In addition to single unit analysis, measurement of immediate early gene (IEG) activation across brain regions provides a means of visualizing the pattern of neural activation resulting from specific behaviors in the intact animal. Detecting changes in the pattern of IEG activation in HPC and DS provides a different level of analysis for identifying changes in neural plasticity associated with learning. Activation of certain IEGs, such as *c-fos* and *zif268* (Krox-24, NGFI-A, EGr1, and ZENK) has been implicated during the consolidation of memory (Hall, Thomas, & Everitt, 2001; Huff et al., 2006; Weitemier & Ryabinin, 2004). The degree to which a structure displays differential amounts of IEG activation during different learning paradigms, such as place or response learning, could indicate their relative contribution to behavior. HPC IEG expression is induced after spatial learning (Guzowski, Setlow, Wagner, & McGaugh, 2001; Vann, Brown, Erichsen, & Aggleton, 2000).

Traditional views of multiple memory system function, originating primarily from lesion studies, hypothesize that disparate neural systems operate independently to regulate behavior. This perspective appears at odds with the apparent collaboration among systems based on similarities in neural processing. However, it could be that differences in responsiveness to neuromodulators such as dopamine underlie the distinct mnemonic functionality of different regions. If this were the case, IEG activation could likewise be differentially regulated by neuromodulatory activity. Accordingly, it would be expected that HPC should be selectively active during HPC-dependent tasks, while DS should become selectively active during striatal-dependent tasks. Consistent with this prediction, Colombo, Brightwell, and Countryman (2003) demonstrated that differences in HPC c-Fos expression 1 h after T-maze training correlated with a place strategy employed during a post-criterion probe trial. However, response strategy use did not induce the expected analogous increase in c-Fos in DS. Nevertheless, the observed structure-specific changes in IEG response to different behavioral paradigms could support the participation of these regions in separate memory systems.

The failure to find differential IEG activation in DS in previous studies may have been a result of insufficient task demands, or the potential differences were masked by simultaneous activation within HPC and DS. It is possible that explicit response testing could increase DS IEG expression above threshold for measurable activation, or sufficient response learning could cause IEG expression to diminish in other regions while DS levels remain constant. This study sought to determine whether explicit place and response testing on the radial maze would lead to differential IEG activation in the HPC or DS, respectively. To accomplish this, a new behavioral paradigm was developed to allow validation of IEG activation related to learning a specific cognitive strategy. Rats were trained on either a place or response task, and HPC and DS IEG activation was compared.

It was uncertain what the temporal pattern of activation of Zif268 and c-Fos immunoreactivity (IR) would be. Typically, IEG protein products are quantified approximately 1–2 h after exposure to experimental conditions (Chaudhuri, Nissanov, Larocque, & Rioux, 1997; reviewed in Guzowski et al., 2005; Morgan, Cohen, Hempstead, & Curran, 1987). The tasks used in this study require 60–90 min of testing. Therefore, one possibility is that structures are engaged at the onset of testing, with peak expression occurring shortly after the 60–90 testing session. Alternately, reaching behavioral criterion, or accurate levels of performance, may signal optimal activation of the brain regions engaged during learning and trigger IEG activation at this timepoint. Subsequently, peak expression would occur 90 min after behavioral criterion had been reached. Therefore, the present study compared c-Fos-IR and Zif268-IR in DS and HPC at two different timepoints, 30 or 90 min after animals reached behavioral criterion.

2. Methods

2.1. Animals

Subjects were male Long-Evans rats ($N = 32$; Charles River, Raleigh, NC) individually housed within a temperature-controlled environment (21 °C) in Plexiglas cages and maintained on a 12-h light–dark cycle. All behavioral testing occurred during the light portion of the cycle. Food and water were available ad libitum for 7 days upon arrival. Subsequently, prior to testing, animals were handled daily and food was restricted to maintain animals at 80% of their initial ad-lib weight. Animals had free access to water throughout the experiment. All methods described were in compliance with the University of Washington Institutional Animal Care and Use Committee and National Institutes of Health guidelines for the care and use of animals in research.

2.2. Behavioral testing

2.2.1. Apparatus

All animals were trained on a semi-automated modified eight arm radial maze, consisting of eight black Plexiglas runways (58 × 5.5 cm) that extended from a central platform (19.5 cm in diameter) and raised to a height 79 cm from the floor. Each runway was hinged in the center so that each arm could be raised or lowered independently. Place testing required a plus maze configuration. A rotating T-maze configuration was utilized for response testing, summarized in further detail below. The maze was

enclosed within a circular black curtain (10″ in diameter) hung from an overhead track. For place testing and control animals in the cue condition, extramaze visual cues were placed on the curtain at random locations.

2.2.2. Habituation

Past research has indicated that it is important for studies examining IEG activation to include sufficient controls for possible effects of stimulus or environmental novelty (e.g. Jarvis, Mello, & Nottebohm, 1995; Zhu, Brown, McCabe, & Aggleton, 1995). In the present study, that habituation phase was conducted to expose animals to all possible reward locations in the environment, thereby ensuring that subsequent IEG activation during testing could not be attributed to any perceived "novelty" of receiving reward in a new location. All animals received 8 days of habituation to the maze apparatus and chocolate milk reward. Each day, only one randomly chosen maze arm was made available. Animals were repeatedly placed on the central platform and traveled to the distal end of the maze arm to receive the chocolate milk reward. After consuming the reward, animals were removed from the maze and placed in an intertrial interval (ITI) box while the maze arm was re-baited. Animals were habituated in this manner until they consistently retrieved reward (at least 15 arm entries in 15 min). Two animals were removed from the study after failing to meet this minimum requirement. For animals that had been randomly assigned to the response learning group, described below, the ITI box location randomly varied across days. In addition, extramaze cues were removed for animals randomly assigned to the response learning group or the control/cueless condition (described below). It has previously been shown that rodents trained on the radial maze have a natural propensity for using extramaze cues to navigate toward food reward even after the development of response strategies (Dale & Innis, 1986; Maki, Beatty, Hoffman, Bierley, & Clouse, 1984). Consequently, extramaze cues were removed for response testing to decrease the likelihood that animals would make spatial associations of the location of reward in relationship to the cues. By the end of the habituation sessions, animals had received exposure to all possible maze arms and corresponding goal locations. Following habituation, animals were trained on either the place or response tasks.

2.2.3. Place testing

A plus-maze configuration was used for place testing during which there were three maze arms as possible start locations and a single goal location (Fig. 1a). Animals began each trial at the end of a randomly selected start arm facing the outer curtain. The same goal location was baited with chocolate milk throughout the testing session. Incorrect responses, or entries into unbaited arms, were recorded and animals were immediately returned to the ITI box for 30 s. Animals were trained in this manner until they had reached a behavioral criterion (8 corrects responses out of 10 trials). After reaching criterion, animals performed a probe trial during which a novel start arm was presented and the four arms used during testing were baited. Selection of the original goal location used during testing was recorded as a correct response during the probe trial.

2.2.4. Response testing

Response testing utilized a rotating T-maze configuration within a 4-arm plus-maze (i.e. four different start locations with goal locations 90° to the left or right of the start location). As was the case during the habituation phase, no extramaze cues were present in the testing environment to reduce the likelihood of any IEG activation from the presence of novel objects. Response testing was divided into two phases (Phases 1 and 2) (Fig. 1b). The Phase 1 consisted of 4 blocks of 10 trials with each block utilizing a single start location. At the beginning and in the middle of each testing phase block (i.e., trials 1 and 6), animals were given a forced-choice trial in which only the start and goal arms were available. Following completion of these forced choice trials, both arms that were 90° to the start location were raised and the animal had to make the correct behavioral response, i.e. make the same 90° turn that was used in the forced choice trial, in order to attain reward. If the rat made an error, they were immediately removed from the maze and returned to the ITI box. After completion of 10 trials from one start location, different start locations were selected for Blocks 2, 3, and 4 of Phase 1. As with Block 1, forced choice trials began each block to remind the animal of the behavioral response required at each start location.

Following Phase 1, animals then began the testing phase (Fig. 1c). Phase 2 was similar to place training except that start arms were randomly selected for each trial. Regardless of the start location, rats had to make the same turn made during Phase 1 (right or left) to reach the goal location. Animals were tested until a sliding criterion of 80% correct responses (8 correct arm entries 10 trials) was reached. Upon reaching criterion, animals performed a probe trial during which a novel T-maze configuration with a new start location was used to determine if animals continued to make the trained response.

2.2.5. Control animals

Control testing was conducted for a subset of animals in order to control for possible IEG activation resulting from motor activity or reward. Control animals were habituated to the testing environment as described previously for place and response animals. During control testing, animals were placed on the center of the maze and allowed access to a single randomly selected maze arm. Animals were yoked to the average number of trials performed as well as the average number of reinforced and unreinforced arm entries experienced by either place or response animals. Accordingly, the chocolate milk reward was randomly omitted from the end of the maze arm for certain trials. After animals had reached the end of the arm on a single trial, and consumed the reward when available, they were returned to the ITI box for a brief interval. The next trial began when animals were again placed on the center platform. Consequently, since control animals had similar amounts of reinforcement and motor activation as place and response animals, IEG activation in the learning groups could be more directly related to acquisition of a specific strategy.

2.3. Immunohistochemistry

c-Fos and Zif268 immunoreactivity (c-Fos-IR and Zif268-IR) was quantified at one of two timepoints following behavioral criterion. Typically, peak c-Fos-IR is measured 90–120 min after either pharmacological or behavioral manipulation (Chaudhuri et al., 1997; reviewed in Guzowski et al., 2005; Morgan et al., 1987). While c-Fos-IR is greatest immediately after learning a spatial task, differential increases in c-Fos-IR in HPC were reported 1 h after testing (Colombo et al., 2003). Pilot data revealed that animals required roughly 60 min to complete response testing. If IEG activation was initiated at the beginning of testing, then peak expression would be expected to occur 30 min after the completion of testing. If IEG activation is more directly linked to the behavior exhibited after learning, then peak expression would occur 90 min after criterion is attained. Therefore either 30 or 90 min after criterion was reached, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with phosphate-buffered saline (PBS) and then phosphate-buffered formalin. Brains were post-fixed in phosphate-buffered formalin for several days. Examinations were focused upon brain areas shown previously to be involved in either place or response learning (McDonald & White, 1993; Packard & McGaugh, 1996).

Fifty micrometer coronal sections throughout the regions of interest were processed for c-Fos-IR and Zif268-IR using polyclonal antibodies for c-Fos or Zif268 (Santa Cruz Biotechnology, Santa Cruz, CA) and the standard avidin-biotin complex/3,3′-diaminobenzidine with nickel chloride (ABC/DAB; Vector Laboratories, Burlingame, CA) technique detailed below. Slices were rinsed (3× PBS), incubated for 20 min in 0.3% hydrogen peroxide in absolute methanol to quench endogenous peroxidase. Subsequently, tissue was incubated for 1 h in 3% normal goat serum in PBS. Slices were then transferred to the primary antibody which consisted of either 1:20,000 c-Fos or 1:8000 Zif268 polyclonal rabbit IgG. After incubating 48 h at approximately 4 °C in the primary antibody, slices were the rinsed (10× PBS, 1h) and processed with ABC/DAB to visualize the presence of c-Fos-IR and Zif268-IR. The sections were then mounted on microscope slides and counterstained with neutral red. c-Fos- and Zif268-IR was quantified in bilateral samples within 3–50 μm slices encompassing the region of interest (Fig. 2). The experimenter conducting the c-Fos and Zif268 counts

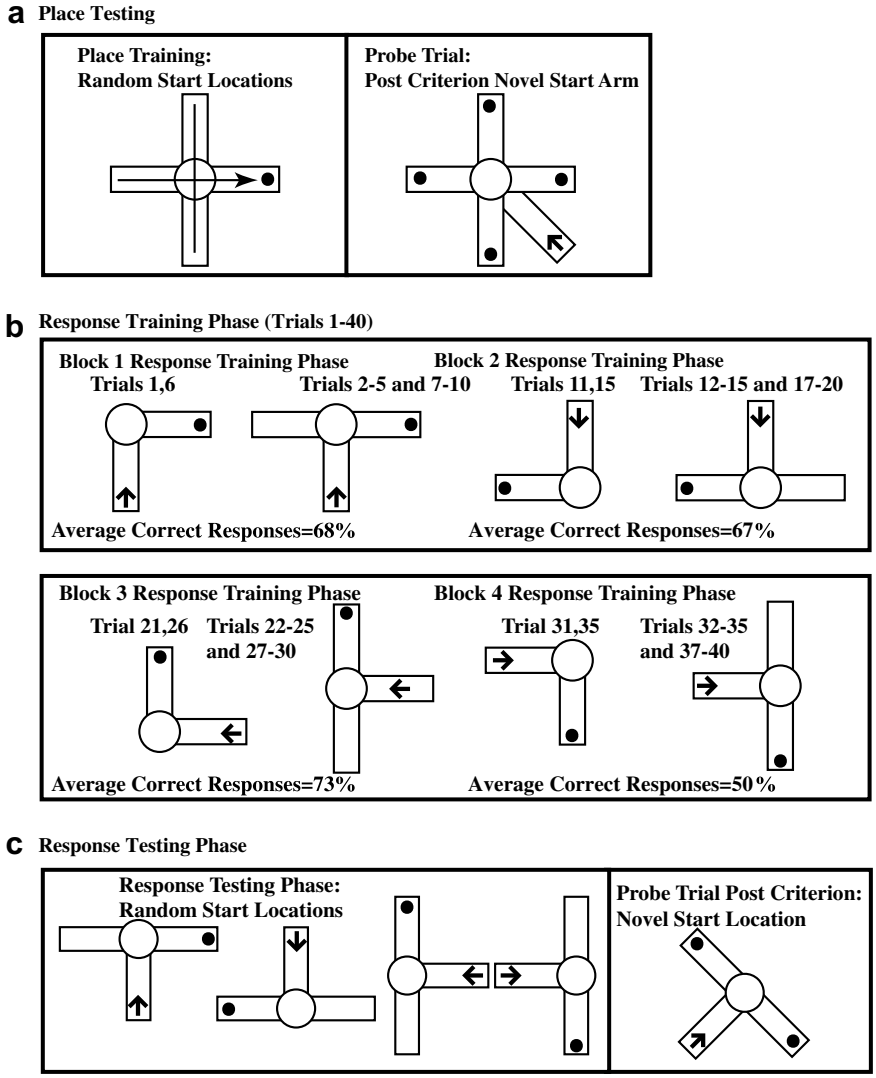

Fig. 1. (a) Schematic of place testing on the plus-maze. Circle represents the goal location. Arrow indicates the randomly presented start location at which the animal is placed at the beginning of each trial during either testing or the probe trial. After reaching criterion, animals performed a probe trial from a novel start arm and entries to all maze locations were rewarded. (b) Schematic of the four blocks of Phase 1 of response testing. Each block consisted of ten trials from a single start locations indicated by arrows. Forced-choice trials were presented as the first trial of each block during which only the start and goal arms were available. (c) Schematic of Phase 2 of response testing during which start locations varied randomly. After reaching criterion, animals performed a probe trial from a novel location and both incorrect and correct responses were rewarded.

was blind to the experimental condition. Atlas coordinates (anterior–posterior relative to bregma) for the sections analyzed began approximately +1.45 to +0.95 mm anterior of Bregma for dorsal striatum and −2.85 to −3.70 mm posterior of Bregma for dorsal hippocampus. The coordinates for these regions were selected based on unit recordings from these areas conducted in this laboratory (Gill & Mizumori, 2006; Mizumori, Ragozzino, & Cooper, 2000; Yeshenko et al., 2004).

2.4. Stereological analysis

A computerized image analysis system (Neurolucida, MicroBrightfield; Colcester,VT) was used for c-Fos and Zif268 quantification. Using the optical dissector method, c-Fos-IR and Zif268-IR was quantified in the dorsomedial and dorsolateral regions of the dorsal striatum and dentate gyrus. Fig. 2a and b illustrate the unbiased optical dissector frame that was superimposed over each sample to delineate the precise sample area to be quantified. Given low levels of c-Fos-IR in area CA1 of the hippocampus, only Zif268 was measured in this region (Fig. 2b). The Swanson (2004) rat brain atlas was used to identify areas to be analyzed.

c-Fos and Zif268-IR were quantified in 3 samples bilaterally within 3–50 μm slices encompassing the region of interest. Sections were chosen on the basis of the locations of anatomical landmarks within the sections. Neurons positive for c-Fos-IR or Zif268-IR were defined as cells with nuclei in which the solid reaction product covered at least half of the nucleus. To account for tissue processed in assays at different times, IEG counts within each region were standardized to *z*-scores.

2.5. Data analysis

Behavioral data for place and response groups were analyzed using repeated measures MANOVAs (Pillai's Trace) with blocks as repeated measures and probe trial response as the between-subjects factor. c-Fos- and Zif268-IR was analyzed using repeated measures MANOVAs with structures as the repeated measures and learning group, timepoint, and probe trial response as the between-subject factors. To evaluate relative IEG-IR between DS and HPC, ratios were calculated using the raw IEG counts for c-Fos and Zif268 from each structure. For c-Fos, ratios were calculated for DM:HPC-DG and DL: HPC-DG. For Zif268, ratios were determined

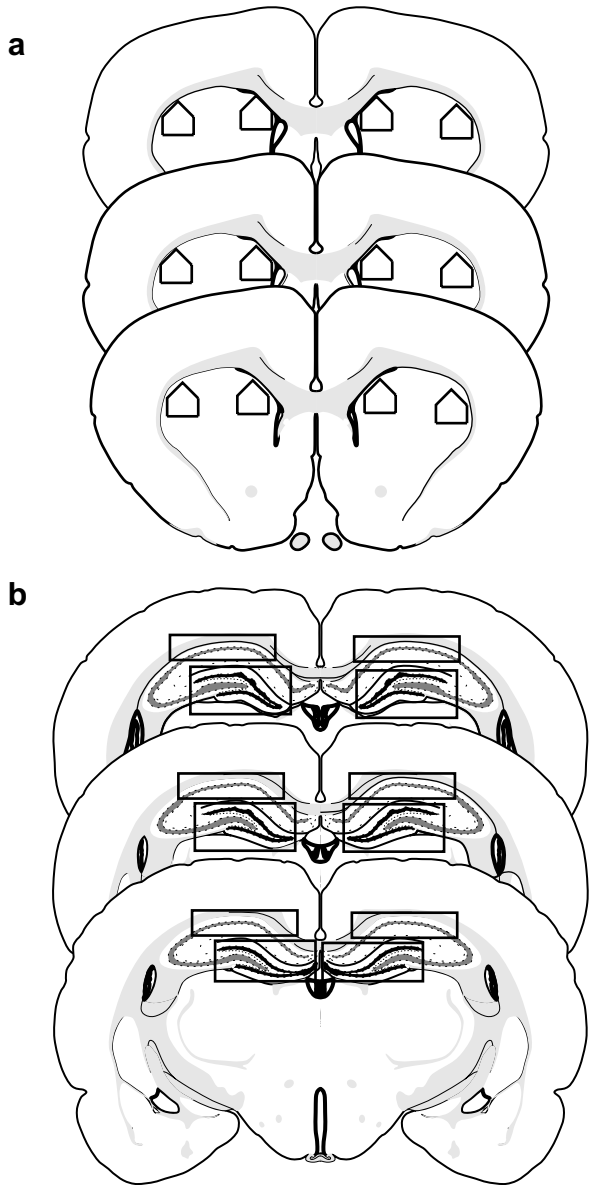

Fig. 2. Diagram of coronal sections illustrating regions quantified in dorsal striatum (a) and hippocampus (b) (Modified from Swanson, 2004). Approximate sample size for dorsal striatum is represented by ⌓. Regions quantified in hippocampus are delineated by ▭, with the upper boxes in each section outlining CA1 and the lower boxes indicating the dentate gyrus. Both the upper and lower blades of the dentate region were quantified.

for DM: HPC-DG, DL: HPC-DG, DM:HPC-CA1, and DL: HPC-CA1. Larger ratio values would indicate greater DS activation relative to HPC. ANOVA was used to determine the effect of strategy on these ratio values.

Correlational analysis was used to assess the relationship between IEG expression and number of trials performed or amount of time on the maze and the Spearman correlation coefficient, Rs, was reported.

3. Results

3.1. Learning rates and probe trial performance

Learning rates for place and response animals were determined separately based on performance during the probe trial at the completion of testing. A majority of place-trained animals ($n = 12/13$) performed correctly during the probe trial and returned to the correct goal location when a novel start arm was presented. These animals also required an average of 35.31 ± 2.63 (mean $\pm$ SEM) trials to reach criterion. Learning curves were constructed for place animals by calculating the proportion of correct responses for successive blocks of five trials (Fig. 3a). Learning curves for response animals were constructed in a similar manner except that response accuracy during the Phase 1 was calculated for four blocks of eight nonforced-choice trials (Fig. 3b). Response accuracy for place testing and Phase 2 of response testing was calculated as the proportion of correct responses for each block of five trials. In contrast to the probe trial accuracy of place learners, there was a subset of response-trained animals ($n = 6/16$) that failed to make the same response during the probe trial that was learned during the testing phases. A one-way ANOVA was conducted to determine if the performance during the probe trial corresponded with the overall number of trials performed prior to criterion. Response animals that passed the probe performed significantly fewer trials (50.60 ± 1.06) before reaching criterion during the learning phase than response animals that failed the probe (61.67 ± 4.77; $F(1,14) = 8.23$; $p < .05$). Despite this difference in the number of trials performed prior to criterion, response animals did not differ in the overall amount of time spent on the maze ($F(1,14) = 1.22$; $p > .05$). Any difference in the amount of IEG-IR between response animals is unlikely to be due to differences in the amount of time in the testing environment.

To establish the extent to which performance improved across the testing session, the proportion of correct responses was compared across blocks of trials. Due to variability in the number of trials performed before criterion, repeated measures analysis of the proportion of correct responses was restricted to only the first 6 blocks of testing in which all animals contributed behavioral data. For place learners, the proportion of correct responses changed significantly across blocks ($F(5,55) = 7.87$; $p < .001$). Since only a single place learner made an incorrect response during the probe trial, direct statistical comparisons with those animals that performed accurately during the probe trial was not possible. For response learners, performance during the probe trial was used as a factor in the repeated measures analysis. Like place learners, response learners also exhibited significant increases in the proportion of correct responses across testing ($F(5,70) = 2.73$, $p < .05$). Furthermore, there was also an interaction effect between probe trial performance and the proportion of correct responses across testing ($F(5,70) = 2.94$; $p < .05$). This interaction suggests that response animals that performed correctly during the probe trial made fewer errors during later blocks, 4 and 6, of acquisition.

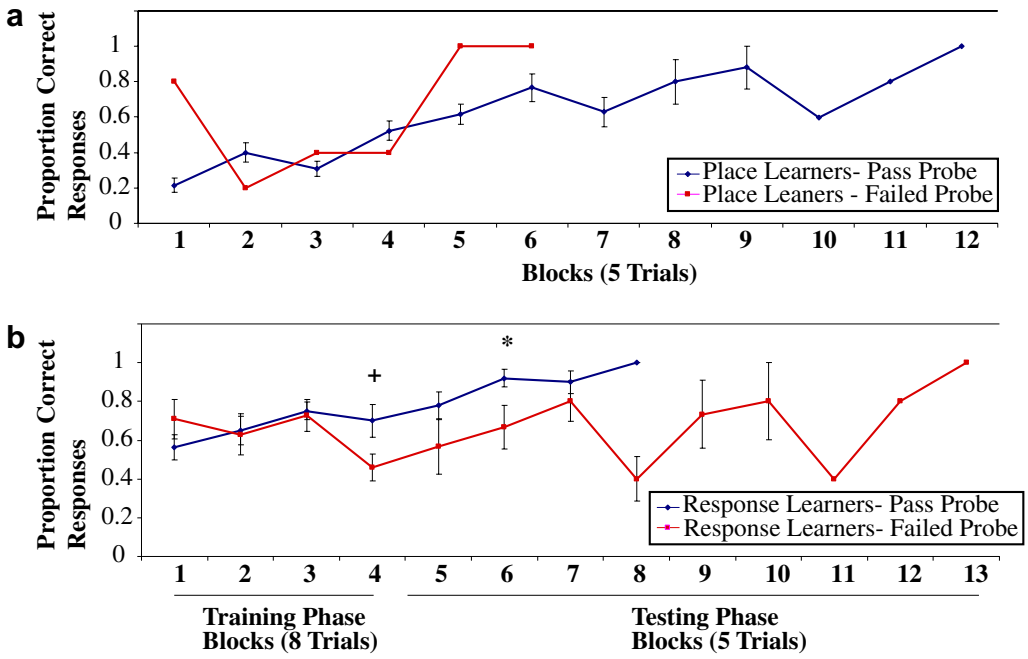

Fig. 3. Summary of performance during place and response testing. (a) There was a significant increase in the proportion of correct responses across blocks (5 trials) of place testing. Only a single place learner (red line) failed to return to the correct location during the probe trial. (b) There was a significant increase in the proportion of correct responses across blocks of response testing. Response animals that made incorrect responses during the probe trial performed significantly more trials before reaching criterion. In addition, during blocks 4 and 6 of response testing, animals that passed the probe trial made fewer errors than animals that failed the probe trial. (+ signifies $p = .07$; * signifies $p < .05$).

3.2. Testing-induced differences in immediate early gene activation

c-Fos-IR and Zif268-IR was quantified in DM, DL and HPC (dentate gyrus) of place-trained, response-trained, and control groups. Zif268-IR was also measured in CA1 of HPC, but due to low levels of c-Fos-IR in this region, CA1 values were not included in the repeated measures analysis and were instead analyzed separately. Since measurements taken from the two control groups, animals run in cueless environment and animals run in standard cued environment, were not statistically different in any region, all control values were pooled within the 30- and 90-min timepoints. Importantly, the two control groups were also yoked to the average number of trials, both reinforced and error, performed by the place and response groups. Consequently, the lack of difference in IEG expression between control groups suggests that any differences seen in place and response animals would not necessarily be the result of disparity in motor activity or amount of reinforcement, but are more likely the related to strategy. Repeated measures analyses of c-Fos-IR and Zif268-IR in the three brain regions were conducted with learning strategy, timepoint, and probe trial performance as between subject factors.

Compared to control animals that received reward without having to utilize a specific strategy, place and response learners should exhibit more c-Fos and Zif268-IR. If animals selectively learned a place or response strategy, then there should be strategy-specific IEG activation in DM, DL, and HPC-DG regions.

3.2.1. Temporal differences in immediate early gene activation

A repeated measures MANOVA comparing c-Fos-IR and Zif268-IR in the dentate gyrus region of HPC, DM and DL regions of DS revealed significant effects of timepoint ($F(2,28) = 14.58$, $p < .0001$), and this varied as a function of strategy ($F(4,58) = 4.39$, $p < .05$). Also, IEG × timepoint × probe interaction effects were observed ($F(4,26) = 2.78$, $p < .05$). CA1 levels of Zif268-IR were not included in this analysis since a lack of c-Fos-IR made it impossible to perform a comparison. Both c-Fos and Zif268 exhibited greater activation 30 min post-criterion compared to levels measured 90 min post-criterion for both HPC and STR. Since protein levels are elevated 90–120 min following a salient event (Chaudhuri et al., 1997; reviewed in Guzowski et al., 2005; Morgan et al., 1987), the elevation of both IEGs soon after testing in this study could indicate that their production was initiated at testing onset and not at the point when behavioral criterion was attained.

Since the place and response groups differed in the number of trials performed prior to criterion, correlational analysis was used to evaluate the possibility that changes in c-Fos-IR and Zif268-IR in each structure were related to the number of trials performed. For DM, DL, and HPC regions, there was not a significant relationship

between the number of trials performed and the amount of c-Fos-IR (DM: $R_s = .26$; DL: $R_s = .16$; HPC-DG: $R_s = .085$, $F = .20$; $p > .05$ for all comparisons). Similar results were obtained comparing the number of trials performed to Zif268-IR in DM, DL, and HPC (Dentate, and CA1); DM: $R_s = .08$, DL: $R_s = .08$; HPC-DG: $R_s = .00$; HPC-CA1: $R_s = .07$; $p > .05$ for all comparisons. The average time spent on the maze was not significantly different between place and response groups (84.64 ± 19.87 and 83.38 ± 18.71 min, respectively). However, correlational analysis was also used to eliminate that possibility that differences in the amount of time spent on the maze were the cause of changes in IEG-IR. Similar results were obtained for time spent on the maze as for number of trials performed. For DM, DL, and HPC-DG regions, there was not a significant relationship between the number of trials performed and the amount of c-Fos-IR (DM: $R_s = .11$; DL: $R_s = .01$; HPC-DG: $R_s = .14$; $p > .05$ for all comparisons). In DM, DL, and HPC (Dentate and CA1), there was also no relationship between time spent on the maze and Zif268-IR (DM: $R_s = .15$; DL: $R_s = .07$; HPC-DG: $R_s = .16$; HPC-CA1: $R_s = .21$; $p >$,05 for all comparisons).

3.2.2. Pattern of hippocampal immediate early gene activation does not differentiate disparate learning strategies

The interaction effects (described above) revealed by the repeated measures analysis suggest that there were differences in IEG activation dependent on either strategy or probe performance. To determine whether these effects were structure-specific, MANOVA comparisons were performed by structure to determine the within-structure effects of learning strategy, timepoint, or probe trial performance. Dentate and CA1 levels of Zif268 both 30 and 90 min post-criterion failed to differentiate the place and response learners from control animals ($p > .05$). The same pattern was seen with c-Fos-IR in the dentate (Fig. 4a and b). Overall, it would appear that neither place nor response testing elicited IEG activation above control levels within HPC.

3.2.3. Pattern of striatal immediate early gene activation distinguishes place and response testing as well as probe performance

Zif268-IR in both DM and DL regions of the striatum failed to distinguish place and response learning strategies from control behaviors. In contrast, differences in DM and DL c-Fos-IR separated learning strategy from control performance. Relative to the control condition, the elevation in c-Fos-IR in response learners was structure and timepoint-dependent. There was a significant increase in c-Fos-IR in the DL, but not DM, region of response learners 30 min post-criterion ($F(2,28) = 4.40$, $p < .02$; Fig. 4a and b). This increase in c-Fos-IR in DL was not seen at the 90 min timepoint.

c-Fos-IR in the DM and DL regions of response learners was dependent on probe trial performance as

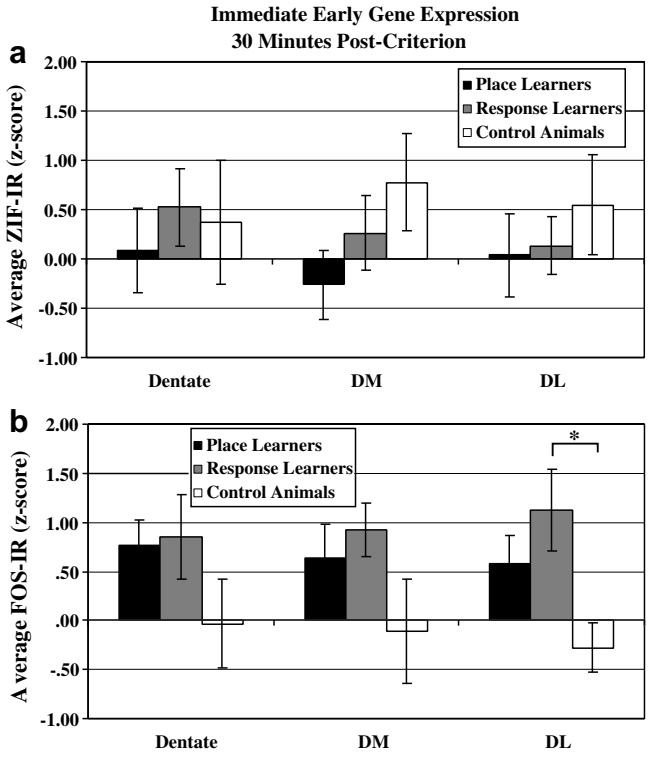

Fig. 4. Bars represent standardized amounts (z-scores) of immediate early gene expression averaged within hippocampus (HPC), dorsomedial (DM), and dorsolateral (DL) striatum 30 min post-criterion for place and response-trained animals and control animals. (a) Overall, the pattern of Zif268 activation in HPC, DM, and DL did not distinguish place and response learning strategies from control testing. (b) Response animals displayed elevated c-Fos activation compared to control animals in DL. c-Fos expression in HPC, DM, and DL of place animals was not significantly different from controls. (* signifies $p < .05$).

evidenced by a significant strategy × probe interaction ($F(1,28) = 2.63$, $p < .05$ and $F(1,28) = 2.32$, $p < .05$, respectively; Fig. 5). Animals that made the appropriate behavioral response during the probe trial displayed more c-Fos-IR in DM and DL compared to animals that failed to make the correct response. Therefore, not only did response animals that probed correctly learn at a faster rate than those that did not probe correctly, they also exhibited more c-Fos-IR in the dorsal striatum. Interestingly, HPC-DG c-Fos- and Zif268-IR were not elevated above the control condition in either place or response animals. Accurate probe trial performance was also not associated with increases in HPC c-Fos- or Zif268-IR. Therefore, there was no general increase in IEG due to changes in performance accuracy per se. Rather, the effects were specific to activity within the DS. Figs. 6 and 7 provide representative examples from DS and HPC of individual place, response, and control animals.

Past studies have explored shifts in relative DS and HPC activation based on the ratio of DS to HPC acetylcholine release resulting from extended training on a T-maze or explicit place and response testing (Chang & Gold, 2003; Pych, Chang, Colon-Rivera, Haag, & Gold, 2005). Use

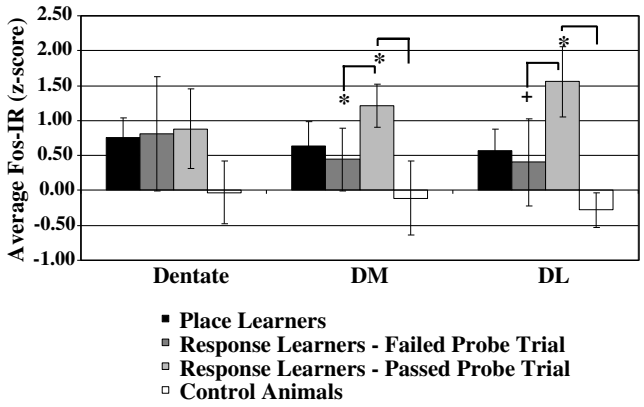

Fig. 5. Bars represent standardized amounts (z-scores) of immediate early gene expression averaged within hippocampus (HPC-Dentate), dorsomedial (DM), and dorsolateral (DL) striatum 30 min post-criterion. Response-trained animals that performed correctly during the probe trial exhibited greater c-Fos immunoreactivity in DL and DM regions compared to control animals. Place-trained animals and response-trained animals that failed the probe trial did not differ from controls (* signifies $p < .05$; + signifies $p = .06$).

of a spatial strategy to perform a task was associated with relatively greater HPC activation, and conversely use of a response strategy, shifted the activation to DS. In the present study, ratios between DM/HPC-DG and DL/HPC-DG for c-Fos-IR were determined. In addition, comparisons for Zif268-IR were made using the following ratios; DM:HPC-DG, DL:HPC-DG, DM:HPC-CA1, DL:HPC-CA1. Any strategy-related differences in relative DS and HPC activation was explored in those animals that passed the probe trial at the completion of training and only at the peak of IEG expression, 30 min post-training. ANOVA was used to determine if there were significant differences between place and response animals in these ratios. Interestingly, for c-Fos-IR, there was a trend approaching statistical significance for response animals to exhibit larger ratio values for DM/HPC-DG and DL/HPC-DG than place animals (Fig. 8; $F(1,10) = 4.27$, $p = .06$ and $F(1,10) = 3.88$, $p = .07$, respectively). This would indicate that response animals had relatively greater DS activation than place animals. Comparisons of Zif268-IR ratios did not yield any differences between place and response animals. (DM/HPC-DG, $F(1,10) = .73$; DL/HPC-DG, $F(1,10) = .31$; DM/HPC-CA1, $F(1,10) = 1.1$; DL/HPC-CA1, $F(1,10) = .91$; all p's $> .05$).

Given the significant differences in the rate of acquisition between response animals that performed correctly during the probe trial and response animals that failed the probe trial, it would be informative to examine any differences in IEG-IR between these two groups. However, given that it has already been established that these two groups differed in the number of trials required to reach criterion, ANOVA was used to determine whether they also differed significantly in the total amount of time spent on the maze. There was not a significant difference in the amount of time spent on the maze between the two response groups (correct probe $= 71.40 \pm 20.35$ min; incorrect probe $= 103.33 \pm 37.82$ min; $F(1,14) = .68$, $p > .05$). Subsequently, it is improbable that any differences in the pattern of IEG-IR observed between these animals is the result of time spent in the testing environment. Instead, disparity in the acquisition of the response task likely account for these changes.

4. Discussion

4.1. Explicit response testing induces c-fos in dorsal striatum early after learning

Response learning on the radial maze-induced differential IEG expression in DS, but not HPC. Effective use of a response strategy, characterized by rapid learning and accurate probe trial performance, was associated with increased c-Fos immunoreactivity (c-Fos-IR) in the DL and DM regions of the striatum. Response testing did not provoke IEG activation beyond control levels in the hippocampus. In addition, the c-Fos-IR induced by response testing was observed relatively early following testing, 30 min post-criterion, suggesting that synthesis actually occurred at the onset of testing.

It has been proposed that activation of DS as part of the cortico-basal ganglia—thalamic circuit is critical during learning situations involving the generation of a new response pattern in a familiar context (Ragozzino, 2003; Ragozzino, Jih, & Tzavos, 2002a; Wise, Murray, & Gerfen, 1996). DS inactivation, via lesions or local anesthetics, usually does not interfere with initial learning of spatial, visual cue or response discrimination tasks, but is devastating on performance during reversal learning (Divac, 1971; Pisa & Cyr, 1990; Ragozzino & Choi, 2004; Ragozzino, Ragozzino, Mizumori, & Kesner, 2002b). In some instances, lesions of the dorsolateral portion of DS can also interfere with initial learning when the number of available visual cues is reduced, as in the present study (Chang & Gold, 2004). Following the extensive habituation procedure in this study, learning to execute the correct behavioral response during response testing likely required additional DS activation, and this was reflected in the c-Fos-IR. Colombo et al. (2003) also found that response animals exhibited elevated levels of phosphorylated cAMP response element-binding protein (pCREB) in DS, an upstream constitutive transcriptive factor for IEG activation. However, there was not a corresponding dissociation between place and response animals in c-Fos-IR in DS. This indicated that there is not always complete correspondence between levels of pCREB and subsequent IEG activation. It is possible the availability of spatial cues in this study lessened the degree of DS activation due to a tendency for animals to rely on spatial cues even when a response strategy is developed (Dale & Innis, 1986). Subsequently, the sustained DS activation required for IEG activation was not attained.

Place testing failed to induce increases in c-Fos or Zif268-IR in either DS or HPC. It is possible that the

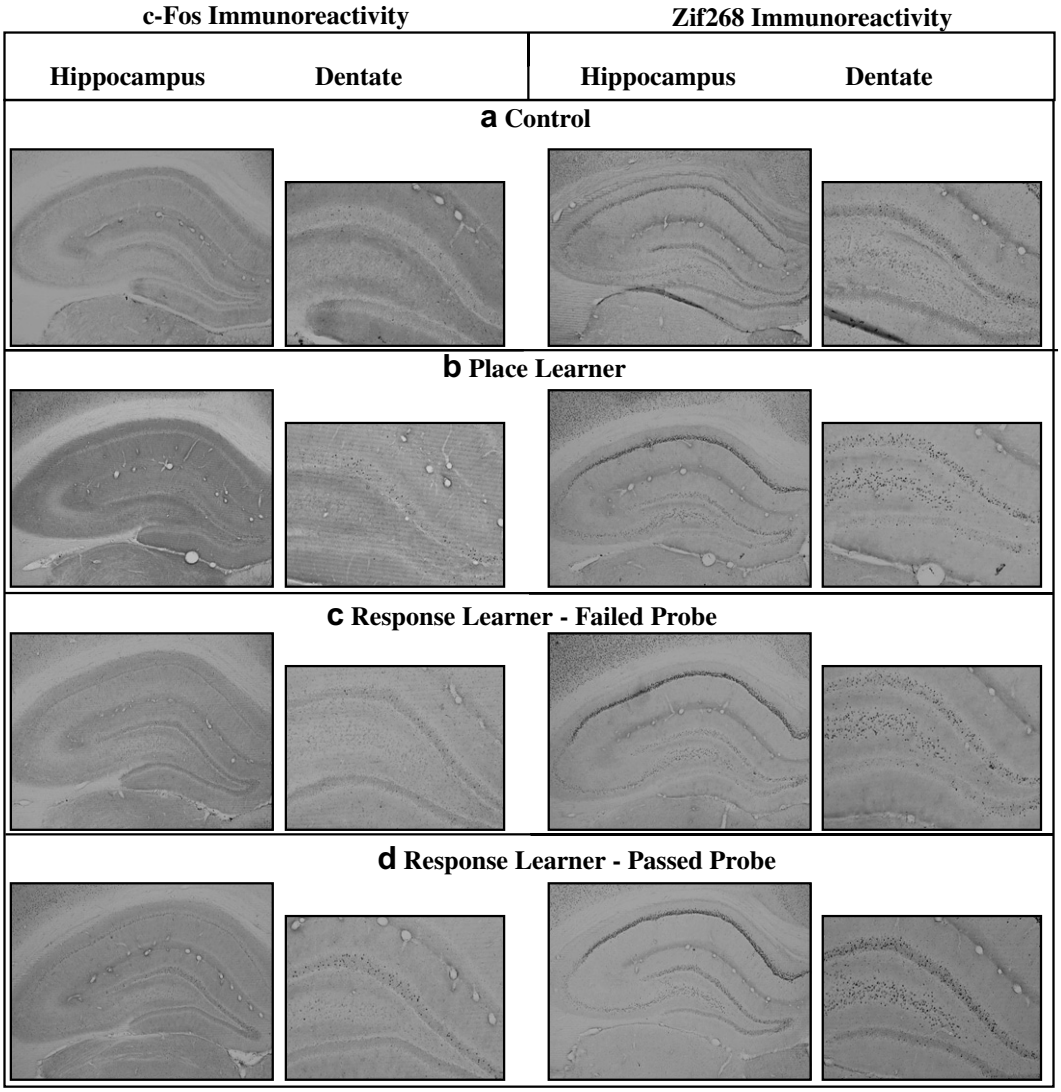

Fig. 6. Examples of c-Fos (left columns) and Zif268 (right columns) immunoreactivity in dorsal striatum from individual control (a), place-trained (b), response-trained/failed probe trial (c), and response-trained/passed probe trial animals (d).

habituation protocol used in this study enabled animals to automatically acquire and consolidate a spatial representation of the possible goal locations. The automatic acquisition of such a spatial representation may be an inherent component of the function of HPC (Morris & Frey, 1997). Consequently, place testing, and to some extent the control testing, might simply reactivate spatial representations that were already acquired during habituation. Another explanation for the lack of increased IEG activation in HPC of place learners could be that rats acquired a spatial strategy during habituation. Expression of a previously acquired strategy may not be sufficient to induce differential HPC IEG activation.

The difference in the number of trials performed by place and response learners prior to reaching criterion would suggest a difference in the level of difficulty. It has previously been shown that during repeated training on the T-maze, animals initially exhibit a spatial strategy while expression of a response strategy occurs much later in training (Chang & Gold, 2003). Response testing in the present study was modified (absence of extramaze cues and Phase1 of testing) to account for this apparent difference in difficulty and allow animals to reliably learn a response pattern in a single testing session. Importantly, IEG-IR was not correlated with the number of trials performed by either place or response learners or the amount of time spent on the maze. Therefore, the changes in IEG activation could not be attributed simply to the level of difficulty of response learning.

A recent study examined regional differences in c-Fos or c-Jun in DS and HPC following testing in two water maze tasks (Teather, Packard, Smith, Ellis-Behnke, & Bazan, 2005). The authors report testing-induced increases, both spatial and cued, in c-Fos in several areas of HPC above levels of caged controls. The most provocative increase occurred in CA1 where spatially trained and control

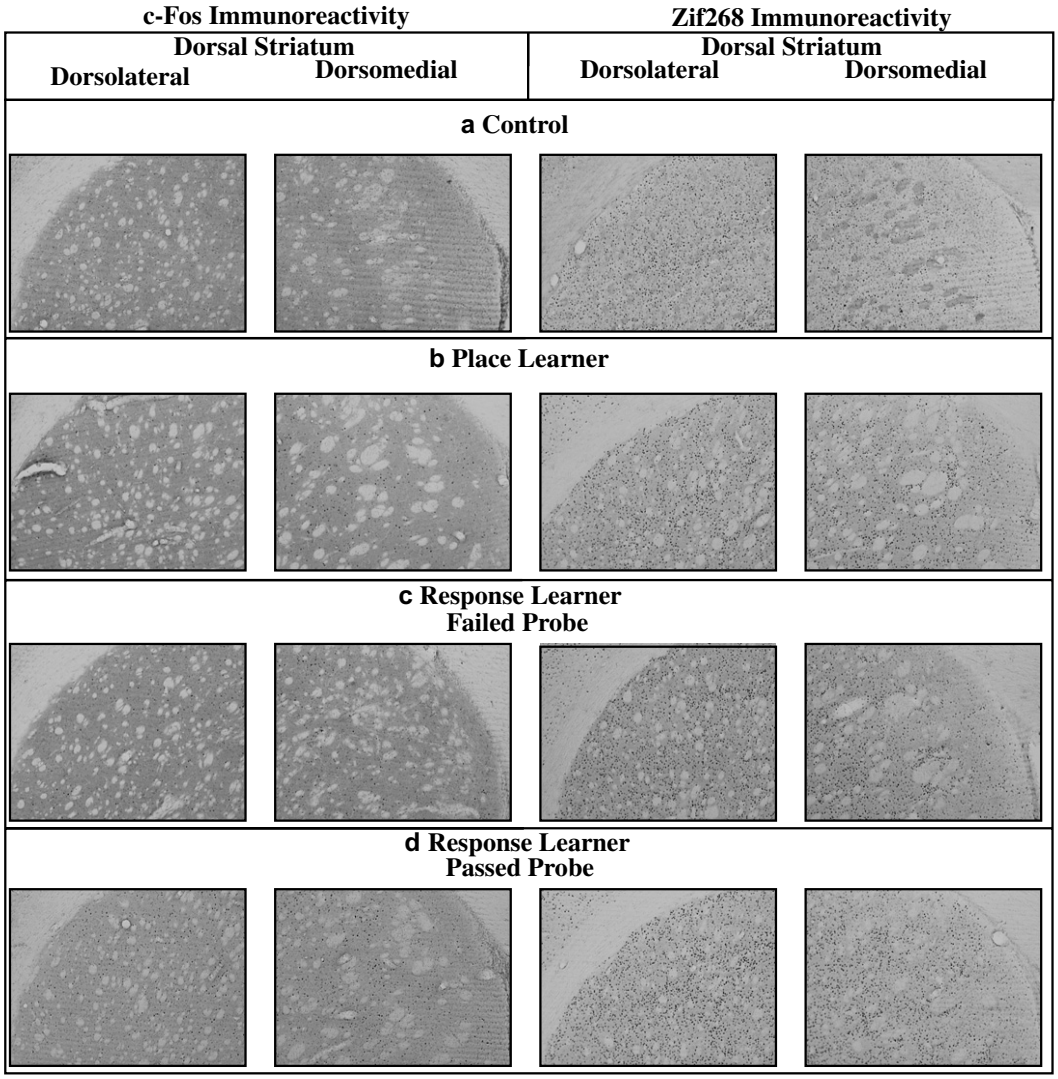

Fig. 7. Examples of c-Fos (left columns) and Zif268 (right columns) immunoreactivity in dorsal hippocampus and denate gyrus from individual control (a), place-trained (b), response-trained/failed probe trial (c), and response-trained/passed probe trial animals (d).

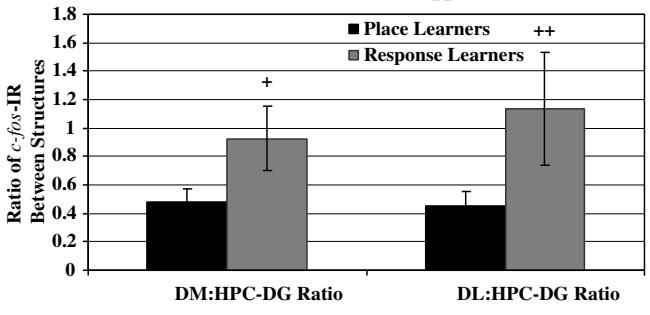

Fig. 8. Bars represent the ratio of c-Fos-IR for DM/HPC-DG and DL/HPC-DG in place and response animals. Ratios for response learners were larger than those obtained for place learners indicating that relative DM and DL activation was greater than HPC-DG for these animals (+ indicates $p = .06$; ++ indicates $p = .07$).

animals, yoked to time spent swimming, displayed significantly greater c-Fos than both caged controls and cued-trained animals. It is unclear why there was not a distinction in level of c-Fos-IR between the spatially trained animals and the yoked controls. Measurement of c-Jun provided clearer spatial-induced increases in CA1 and CA3 that was not related to swimming. There was no difference between spatially trained and cued-trained animals in amount of c-Fos or c-Jun in DS. Interestingly, it was reported that several of the cued-trained animals were distinguished from the rest of their group by qualitative increases of c-Jun in patches in DL. The pattern of IEG expression in DS in this group could correspond to the response-trained animals from this study that performed correctly on the post-testing probe trial.

Importantly, there were several differences in the testing paradigm utilized by Teather et al. (2005) and that which was employed in the present study. First, IEG expression was only measured 90 min after completion of testing. It is possible that by not examining expression 30 min after testing, as in the present study, any differential expression in DS was overlooked. Additionally, the behavioral testing

described by Teather et al. (2005) for the spatial and cued-tasks differed in the amount of time animals were exposed to the testing environment. There was also no significant difference in the number of trials performed by the two groups, suggesting that acquisition of this task in a single session is roughly equivalent.

A third explanation for the finding that place testing did not differentiate c-Fos or Zif268 expression is that place learning activates a different (but perhaps overlapping) subset of cells from those engaged during habituation. This level of change may not be reflected in the simple quantification of protein expression. Indeed, HPC place fields will change their firing properties during new learning or reorganize in response to contextual changes without changes in mean rate (Gill & Mizumori, 2006; Mizumori, Cooper et al., 2000; Mizumori, Ragozzino, Cooper, & Leutgeb, 1999; Smith & Mizumori, 2006). Such alterations in neuronal firing properties may not coincide with activation of c-Fos during learning. However, it has been shown that activation of a different IEG, *Arc*, is linked to some degree to the behavioral history of an animal in a nonlearning situation involving repeated exposures to a familiar testing environment over many sessions (Guzowski et al., 2006).

Guzowski et al. (2001) measured HPC changes in *c-fos*, *zif268*, and *Arc* RNA resulting from a testing procedure similar to that used in the present study during which animals were explicitly trained to use either a spatial or response strategy to locate an escape platform in a water maze. Relative to caged-control animals, there was significant elevation in the RNA of all three IEGs in the HPC resulting from place or response testing in the water maze. However, there was no difference between place and response animals in HPC IEG expression, suggesting that learning either strategy caused significant and comparable IEG activation in HPC. Colombo et al. (2003) measured both HPC and DS c-Fos protein levels after animals were trained on a T-maze task and the strategy employed by the animal was assayed during a probe trial at the completion of testing. Immediately after testing, HPC c-Fos-IR was comparable between place and response animals. In contrast to the results reported by Guzowski et al. (2001) and our findings, Colombo et al. (2003) was able to discern HPC differences in c-Fos activation 1 h after testing resulting from spontaneous place- and response-strategy selection on the T-maze. One potential cause for the discrepancy may be the fact that unlike the present study, Colombo et al. (2003) did not standardize c-Fos-IR relative to control expression. Standardizing in this way would normalize IEG counts relative to any IEG-activation that was not specifically related to cognitive demands, but more likely the result of motor activity or amount of reinforcement. In addition, the more complicated testing procedure in this study entailing multiple start and goal locations may have induced greater HPC activation in animals trained to perform the response task.

A challenge before us is that there is not always correspondence between behavioral impairments caused by lesions, single unit responses during learning, and IEG activation during performance of the same behaviors. HPC-dependent trace fear conditioning does not induce HPC c-Fos levels above control (Weitemier & Ryabinin, 2004). Indeed, delay fear conditioning, a task which does not require an intact HPC, in the same study resulted in c-Fos increases in CA3 and the dentate. While HPC *Arc* expression after exposure to a novel spatial environment correlates roughly to place cell activation during single-unit recordings, there is not always correspondence between actual neural firing patterns and IEG activation (Chawla et al., 2005). HPC neurons can exhibit learning-related changes in activity following auditory fear conditioning, but not differential *zif268* activation (Hall et al., 2001; Rorick-Kehn & Steinmetz, 2005).

Despite the lack of evidence linking changes in IEG activation with changes in neural firing patterns that occur during learning, the results of studies utilizing antisense oligonucleotides to interfere with normal *c-fos* or *zif268* synthesis support the requirement of IEG products in establishing or maintaining memory traces. Genetic deletion of *zif268* can interfere with long-term memory formation following succinct testing scenarios (Bozon, Davis, & Laroche, 2003; Jones et al., 2001). In addition, *zif268* antisense can block the reconsolidation of contextual fear memories when infused into dorsal HPC (Lee, Everitt, & Thomas, 2004). Antisense *c-fos* in the amygdala can impair long-term conditioned taste aversion memory while sparing acquisition and short-term memory (Lamprecht, Hazvi, & Dudai, 1997).

4.2. A possible mechanism for selective activation as a function of strategy use

Activation of the various IEG's can be accomplished via multiple neurotransmitter systems such as acetylcholine (ACh) or dopamine. Pharmacological treatments that engage cholinergic and dopaminergic receptors can cause increases in activation of c-Fos and Zif268 (Dragunow, 1996; Hu, Liu, Chang, & Berg, 2002; Moratalla, Vickers, Robertson, Cochran, & Graybiel, 1993; Thiriet, Zwiller, & Ali, 2001).

Transient changes in ACh levels during learning can be used to predict the strategy employed. As animals engage in HPC-dependent behaviors, there are observable increases in ACh release in HPC (Fadda, Melis, & Stancampiano, 1996; Ragozzino, Pal, Unick, Stefani, & Gold, 1998; Ragozzino, Unick, & Gold, 1996). With continued testing on a standard T-maze task, animals will switch from relying on a spatial strategy to a response strategy (Chang & Gold, 2003). This alternation between two proposed independent memory systems is also correlated with changes in HPC and DS ACh levels. Interestingly, HPC ACh levels remained elevated while there was a gradual increase in DS ACh. When animals are explicitly trained to perform a place or response task, a similar pattern is observed of sustained elevation in HPC ACh levels during

both tasks and significantly greater DS ACh levels during response testing only (Pych et al., 2005). The continued elevation of HPC ACh could partially explain why there were no observable differences in IEG expression between place and response learners of this study. Response learning could entail the eventual activation of DS without a decrease in HPC activation. This is consistent with the pattern of c-Fos activation in DL and DM in this study that appeared to be dependent upon accurate performance during the probe trial. In addition, the ratio of c-Fos-IR between DS and HPC-DG supported greater DS activation in response learners.

Currently, there is no direct evidence supporting transient fluctuations in dopamine efflux in HPC and DS corresponding to activation of different memory systems. However, selective destruction of dopamine signaling in HPC and DS can interfere with performance of HPC- or DS-dependent tasks (Da Cunha et al., 2003; Faure, Haberland, Conde, & El Massioui, 2005; Florio, Capozzo, Nisini, Lupi, & Scarnati, 1999). In addition, Parkinson's patients display similar impaired performance during reversal learning tasks as animals with lesions to DS (Flowers & Robertson, 1985; Robertson & Flowers, 1990). It is possible then that changes in dopamine signaling during learning, similar to those observed for ACh, could provide a means of regulating the influence of a given neural structure or system on behavior (Mizumori et al., 2004). It has been previously proposed that phasic bursts by dopamine cells correspond to positive reinforcement and subsequent activation of cortico-basal ganglia–thalamic pathways leading to a behavioral response (Frank, Seeberger, & O'Reilly, 2004; Suri & Schultz, 1998). Conversely, actions that are not rewarded, or events that are perceived as aversive, can actually cause dips in the baseline level of dopamine supporting inhibition of inappropriate behavioral responses.

It is unclear how dopamine signals within HPC contribute to learning situations during which specific behavioral responses must be linked to environmental or contextual variables. Changes in D1-receptor activation in HPC might be essential for incorporating novel information or changes in a familiar environment (Lisman & Otmakhova, 2001). The gating influence of D1-receptors is revealed in single-unit recordings from HPC as animals perform well-learned spatial tasks. Following manipulations of the testing environment, such as imposed darkness, HPC neural representations display greater instability with combined D1-antagonist treatment (Gill & Mizumori, 2006).

4.3. Relationship between learning and immediate early gene activation

4.3.1. Neural activation and multiple memory systems

The nature of the participation of HPC and DS in different memory systems remains unclear. While the selective effects of lesions on learning suggest anatomically separate memory systems, evidence obtained from single-unit studies, and some studies of IEG activation, indicate potential similarities in the neural responses across different types of learning.

Clayton (2000) described the activation of different IEGs as part of a genomic action potential (gAP) involved in determining whether certain memories are consolidated. The convergence and interaction of different transduction pathways means that small changes in IEG can have dynamic effects on plasticity-related protein synthesis. Subsequently, even transient changes in IEG activation can act as a molecular switch for memory consolidation, ensuring that even brief events are remembered. More importantly, according to Clayton, IEG induction is part of a process vital for resolving ambiguity in contexts involving a high degree of unpredictability.

Simultaneous recording of individual place cells in HPC and DS show that neural responses to contextual changes are comparable (Mizumori, Ragozzino et al., 2000; Yeshenko et al., 2004) in both regions regardless of whether animals perform a HPC-dependent place task or a DS-dependent response task. However, the similarities in the reorganization of spatial firing of these neurons did not extend to the processing of egocentric movement. The velocity-tuning of HPC neurons, but not DS neurons, was more sensitive to disruption by changes in the visual environment selectively during place learning. Thus, while there are similarities in the information encoded in both regions, there can be subtle differences in the response to manipulations. Therefore, findings of correlations between neural activity and IEG expression may depend on which measure of neural change is used. Unlike single-unit activity which may represent recent neural processing, the gAP of IEG induction may discriminate between activity patterns across extended periods. Nevertheless, significant changes in IEG activation may reflect shifts in the relative activation (at the population level) of one structure over another. Elevated IEG in DS but not HPC (as in this study) may indicate a stronger striatal output to behavioral expression systems during response learning compared to HPC. HPC as a whole appears to be engaged during active navigation regardless of the task, although the specific combination of activated HPC neurons may vary depending on task or context change (Gill & Mizumori, 2006; Smith & Mizumori, 2006; Yeshenko et al., 2004).

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