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Head west or left, east or right: interactions between memory systems in neurocognitive aging

Inês Tomás Pereira^{a,1}, Michela Gallagher^b, and Peter R. Rapp^{a,*}

^aNeurocognitive Aging Section, Laboratory of Behavioral Neuroscience, National Institute on Aging, NIH, Baltimore, MD, USA

^bDepartment of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA

Abstract

Cognitive aging is accompanied by decline in multiple domains of memory. Here, we developed a T-maze task that required rats to learn competing hippocampal, and striatal navigation strategies in succession, across days. A final session increased demands on cognitive flexibility and required within-day switching between strategies, emphasizing capacities that engage the prefrontal cortex. Background characterization in young and aged rats used a water maze protocol optimized for individual differences in hippocampal integrity. Consistent with earlier work, young adults acquired place strategies in the T-maze faster than response, whereas the opposite was observed in aged rats with impaired spatial memory. The novel result was that aged animals with preserved spatial memory displayed a qualitatively distinct pattern, acquiring place and response strategies equally rapidly, without disruption when switching between them. Subsequent *in situ* hybridization for the plasticity-related immediate-early gene *Arc* revealed that while increasing demands on cognitive flexibility and within-day strategy switching potentially engaged the prefrontal cortex in young adult and aged-impaired rats, *Arc* expression was insensitive in aged rats with normal spatial memory and superior switching abilities. Together, the results indicate that cognitive aging is an emergent property of the interactions between memory systems, and that successful cognitive outcomes reflect a distinct neuroadaptive process rather than a slower rate of aging.

Keywords

Prefrontal cortex; Hippocampus; Memory systems; Arc; Rat; Neuroadaptive aging

1. Introduction

Deficits in capacities supported by the hippocampal system are among the most widely studied features of normal cognitive aging (Burke and Barnes, 2006; Erickson and Barnes,

*Corresponding author at: 251 Bayview Boulevard, Suite 100, Room 9C224, Baltimore, MD 21224, USA. Tel.: 410-558-8195; fax: 410-558-8328. rapp@mail.nih.gov (P.R. Rapp).

¹Present address: Cognitive, Linguistics and Psychological Sciences, Brown University, Providence, RI 02912, USA.

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2003; Foster et al., 2012). The magnitude of impairment varies widely among older individuals in both humans (Grön et al., 2003; Morse, 1993) and animal models (Gallagher et al., 1993), consistent with the view that decline is not an inevitable outcome of growing older and encouraging the search for mechanisms that support positive cognitive outcomes. Although the aim in many studies using carefully optimized, narrowly tuned behavioral testing procedures has been to examine hippocampal memory in relative isolation, it is clear that memory-related systems outside the hippocampus are also vulnerable. Often conceptualized as independent systems operating in parallel, ongoing behavior can also reflect the outcome of complex competitive and synergistic interactions between multiple memory systems (Kim and Baxter, 2001). In a foundational study on the effects of normal aging, for example, Barnes, et al. (1980) demonstrated that, relative to young adults, aged rats with deficits in spatial memory display an enhanced reliance on response learning under testing conditions that allow both types of task solution. These findings are compatible with substantial evidence from young subjects indicating that hippocampal and corticostriatal memory systems often compete for the control of behavior (Packard, 1999; Packard and McGaugh, 1996; Tolman et al., 1946), and that, released from this competitive interaction as a consequence of compromised hippocampal integrity, response learning predominates in aged subjects.

Impairments in executive function mediated by the prefrontal cortex (PFC) comprise another prominent hallmark of normal cognitive aging in both animal models and humans (Banuelos et al., 2014; Bizon et al., 2012; Turner and Spreng, 2012). Similar to the analysis of aging in the hippocampal system, considerable work in this area has focused on performance capacities known to require PFC integrity, including Wisconsin Card Sorting in humans (Esposito et al., 1999; Ridderinkhof et al., 2002), delayed response performance in monkeys (Rapp and Amaral, 1989), and set shifting in rats (Barense et al., 2002; Beas et al., 2013). Current evidence from young subjects, however, also implicates the PFC in cognitive flexibility and the top-down control of interactions between other memory systems. Results from recent lesion and electrophysiological studies, for example, demonstrate that the prelimbic (PrL) and infralimbic (IL) subdivisions of the medial PFC are critically engaged when novel task demands require switching between place and response-based strategies mediated by the hippocampus and corticostriatal circuitry, respectively (Rich and Shapiro, 2007, 2009). Measured under conditions that promote single task solutions and involve limited overlap in key cognitive demands, the effects of aging appear at least partly independent across different memory systems (Barense et al., 2002; Beas et al., 2013; Gallagher and Burwell, 1989; Schoenbaum et al., 2002). However, research designed to explicitly emphasize the interaction between systems and to examine the role of PFC circuitry implicated in mediating these interactions, has received limited attention in studies of neurocognitive aging.

The present study examined how individual differences in hippocampal integrity in aging affect performance under conditions that explicitly induce competition between allocentric spatial navigation and nonhippocampal egocentric response strategies. Behavioral testing also emphasized demands on executive control, mediating the interaction between striatum- and hippocampus-based task solutions. Briefly, young adults (Y) and aged rats with (aged impaired; AI) or without (aged unimpaired; AU) documented deficits in hippocampal

memory successively acquired place and response strategies in a water-based T-maze. The behavioral results revealed qualitatively distinct patterns of performance across groups. Consistent with earlier studies (Barnes et al., 1980), while young animals were biased toward hippocampus-dependent task strategies, learning places faster than response-based solutions, aged-impaired rats displayed the opposite pattern, acquiring corticostriatal response strategies more quickly than places, and indeed significantly faster than young. The more novel finding was that aged rats with preserved hippocampal memory displayed a pattern distinct from both Y and AI, acquiring place and response strategies equally rapidly, with no decrement in performance when switching between conditions. A final task manipulation increased demands on executive control and for the first time in training introduced an unsignaled switch between response and place task contingencies within a single test session, conducted in a timeframe appropriate for evaluating induction of the plasticity-related immediate-early gene *Arc*. This analysis revealed a marked age-related decline in behaviorally-induced *Arc* expression in most regions examined. Moreover, whereas strategy switching led to significant PFC *Arc* induction in both Y and AI rats relative to controls that performed a single strategy before sacrifice, *Arc* expression failed to differ in AU rats in response to demands on executive control. The latter findings suggest a potential release from top-down PFC control over different memory systems in successful cognitive aging, potentially accounting for the superior strategy switching ability observed in AU rats. Together, the results add to growing evidence that positive cognitive outcomes in aging arise from a late-life process of dynamic neural network adaptation, not simply the persistence of youthful processing.

2. Materials and methods

2.1. Subjects

Male Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA) were housed individually in a vivarium at Johns Hopkins University and maintained on a 12-hour light-dark cycle with food and water available *ad libitum*. Animals were examined for health and maintained under specific pathogen-free conditions throughout the experiments. Young animals ($n = 26$) were 6 months old, and aged rats ($n = 42$) were 24–28 months of age at the time of T-maze testing. Two aged animals died during the course of T-maze testing and 4 were unable to perform the motor demands of the task. These animals were excluded from all analyses. All procedures were approved by the Institutional Animal Care and Use Committees of John Hopkins University and the Intramural Research Program of the National Institute on Aging, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral characterization

The integrity of spatial learning was assessed initially using a water maze procedure validated across many previous studies (Gallagher et al., 2003; Gallagher and Pelley, 1988; Haberman et al., 2011; Schoenbaum et al., 2002; Smith et al., 2000). The water maze consisted of a circular tank (1.83 m in diameter), filled with tepid water ($\sim 25^{\circ}\text{C}$) made opaque by adding nontoxic white tempera paint. The escape platform was located 1 cm below the water surface and maintained in a constant location across training

trials. White curtains with conspicuous visual cues surrounded the maze to facilitate spatial navigation. Animals were tested in the water maze to assess spatial memory capacity, according to a protocol described previously (Gallagher et al., 1993). Training was conducted over 8 consecutive days. Rats were tested for 3 trials per day, with intertrial intervals of 60 seconds. With the location of the hidden platform held constant, the start location varied among 4 equidistant points around the perimeter of the water maze. Every sixth trial was a probe test during which the platform was retracted and unavailable for 30 seconds, after which it was raised to allow animals to escape. Learning index scores were calculated as the weighted average proximity to the platform location across probe trials (Gallagher et al., 1993). Based on normative data from a large number of young animals, aged animals with learning index scores greater than 240 were classified as AI, and those scoring under that cutoff, within the range of the Y group, were defined as AU (Fig. 1).

At the end of the 8-day spatial protocol, animals were tested on a 1-session cued version of the task to identify potential sensorimotor and motivational contributions to performance differences between age groups. This assessment consisted of 6 trials with a variable escape location in which the platform was raised and visible above the water surface. Animals impaired in the cued version of the task were excluded from analysis. A notable feature of the behavioral approach is that rats were provided prior experience with both place and response-based approaches before T-maze training.

2.3. T-maze task

2.3.1. Apparatus—T-maze testing was conducted in a second water maze in a room different from the one used for background behavioral characterization. Plexiglas inserts were used to form 4 arms in a plus-shaped configuration, with access to either the north (N) or south (S) arm blocked during training (Fig. 2). The pool was 1.83 m in diameter and filled with water at approximately 25°C, made opaque by the addition of nontoxic white tempera paint. An escape platform positioned 2 cm under the water surface was located at the end of either the east (E) or west (W) arms (Fig. 2A). Arms were approximately 20-cm wide and 80-cm long, and the center choice-point was a square 20 by 20 cm. The transparent Plexiglas inserts, comprising the walls, extended approximately 12 cm above the water surface. Black curtains with large, sharply contrasting white cues surrounded the maze. These cues were clearly distinct from the ones present for initial behavioral characterization.

2.3.2. Place and response learning—Across trials, Y (n = 8), AU (n = 8), and AI (n = 8) animals entered the maze from either the S or the N start and swam to an escape platform located in either the E or W arm, balanced across rats. A choice was recorded when the entire body of the animal, excluding the tail, passed the goal arm entry point. A correct choice was rewarded with escape followed by 15 seconds on the platform at the end of the arm, and a 60 seconds intertrial interval in the holding cage. After an incorrect choice, animals were allowed to swim to the end of the incorrect arm, where they were retrieved and immediately placed in the start arm for the next trial. Self-correction was allowed only during strategy acquisition (described in the following section), defined as an initial incorrect arm choice, followed by a 180° turn before reaching the end of the arm, and

subsequent entry into the correct, opposite goal. These trials were scored as incorrect. Animals were tested for a maximum of 20 trials per day.

A schematic representing the order of strategies trained is shown in Fig. 2A. Subjects first learned a place strategy (place1, either “go East” as illustrated in Fig. 2, or “go West”) in which escape involved navigating from the N or S start arm to a constant platform location in the testing environment (either E or W, counterbalanced across animals within groups). During an initial phase of acquisition, animals entered the maze from the same arm (e.g., N) until achieving 2 consecutive correct choices. Subsequent trials started from the opposite arm (S, in this example) and testing continued until animals again scored 2 consecutive correct trials. Criterion performance was reached when rats performed 6 correct trials in a row, comprising 3 start arm reversals. Animals that failed to reach criterion within the 20-trial per session cutoff were returned to the home cage (HC) and tested using the same protocol the following day. The day after criterion was reached in the initial acquisition phase, we confirmed that a place strategy was well established by continuing training with the start arm order varied pseudorandomly across trials (i.e., equal numbers of starts from the N and S arms with no more than 3 consecutive starts from the same arm). Criterion accuracy during this phase was defined as a minimum of 10 of 12 trials correct (~83%), and in practice, most animals exhibited little or no decrement in performance when the start arm order was randomized. A single strategy per day was enforced throughout acquisition, and on the day after criterion performance on the randomized start arm phase of place1, training on the first response strategy was introduced (Fig. 2A, right). Escape in this case required a consistent body turn at the maze choice point whether entering from N or S (response1, “turn Left”, as shown in Fig. 2, or “turn Right”, in animals initially trained on “go West”). After reaching the initial accuracy criterion with a predefined sequence of repeated start arms (6 correct trials in a row, comprising 3 start arm reversals), training continued the next day with the start arms varied pseudorandomly across trials. The performance criterion at this phase of response strategy training was defined as 10 out of 12 trials correct across 2 consecutive days, confirming that a robust response-based strategy was well established. After reaching the acquisition criteria for response1, subsequent training switched to the place strategy for the location not learned previously (place2, e.g., “go West” for rats that first learned “go East”). The protocol was otherwise the same as for place1. Finally, rats acquired and were tested on retention of the second response strategy (response2, e.g., “turn Right” if they previously learned “turn Left”). By this design, the testing protocol ensured that the goal arms (E and W) were equally rewarded.

To examine the degree of strategy bias in relation to aging, a strategy preference score was computed by subtracting the summed number of trials to criteria for both response strategies from the total number of trials to criterion across the 2 place strategies, multiplied by the total number of trials needed to complete training. By this measure negative values result from faster learning for place strategies, positive values indicate quicker acquisition for response strategies, and the degree of deviation from 0 reflects the strength of the bias.

2.3.3. Error analysis—Adopting an analytic strategy validated in studies using standard T-maze procedures (e.g., Packard et al., 1989), we were able to classify most errors committed during acquisition according to the strategy being followed. Consider the

example of an animal learning the “turn Left” response strategy that correctly executes an S to W turn followed by an N to W error. Incorrect choices of this sort were categorized as errors arising from the use of a place strategy, comprising a return to a previously rewarded escape location (“go West”). By contrast, an error reflecting the use of a response strategy was scored when, for example, an N to E error followed a correct S to W response in a rat learning the “go West” strategy (both are left turns). In this way, the types of errors committed were classified on a trial-by-trial basis throughout acquisition. Only clearly definable errors were classified as following a place or response strategy, and the remainder, of ambiguous or unknown origin (e.g., errors committed on the first trial of the day, or a correct S to W trial followed by an S to E error), were pooled in an “Undetermined” category for analysis.

2.3.4. Within-day switch—Up to this point in training, reinforcement contingencies were held constant within a daily test session, and all switching between strategies occurred across days. On the final test session, however, the same strategy as the previous day was initially enforced, followed by an unsignaled shift to the alternate task strategy (Fig. 2B). Specifically, the first 6 trials in this phase used a pseudorandom start arm order to test retention of the response strategy established the preceding day. A switch to the place variant of testing was then imposed, and training progressed according to the acquisition procedure for place1 and place2 (i.e., with repeated trials from both the N and S start arms to a criterion of 6 consecutive correct trials). Rats were provided a maximum of 20 trials to master the novel within-day switch (WDS). If the criterion was achieved, training on the place strategy continued for an additional 6 trials with the start arm order randomized. Sacrifice occurred 30 minutes after rats first reached the acquisition criterion or at a corresponding time point for rats that failed to achieve criterion. By this design, the final day of training imposed novel demands on cognitive flexibility, and sacrifice was scheduled in a timeframe appropriate for the analysis of regional expression patterns for the immediate-early gene *Arc* (activity-regulated cytoskeleton-associated protein) across multiple memory systems in relation to task performance.

2.3.5. Behavioral controls—To assess basal levels of immediate early gene expression, a set of control animals was sacrificed directly from the HC. We and others (Fletcher et al., 2014; Hartzell et al., 2013) have found that resting, baseline levels of *Arc* messenger RNA (mRNA) expression are equivalent in the young and aged hippocampus, and in the present experiments only young adult HC controls were examined (n = 5). Additionally, to distinguish the effects of well-established place and response learning from activation induced by the cognitive demands of the WDS, other controls received continued training on only one of the task strategies. These animals were tested on the acquisition stages of either a single place (place alone, Y; n = 7, AU; n = 4, AI; n = 6) or response strategy (response alone, Y; n = 6, AU n = 5, AI; n = 5). On the day after achieving the performance criteria, animals returned to the maze and received 20 trials of the well-learned strategy, matching the maximal length of testing in the WDS condition. Single strategy controls were sacrificed at a time-point equivalent to WDS animals, relative to the initial trial of the day.

2.4. Tissue preparation

Animals were deeply anesthetized at the appropriate time point with 5% isoflurane, followed by transcardial perfusion at 35 mL/min with cold phosphate-buffered saline (PBS) for 2 minutes, and ice-cold 4% paraformaldehyde in PBS (pH 7.2–7.4) for 13 minutes. The brains were removed and postfixed in 4% paraformaldehyde in PBS for approximately 24 hours. The tissue was cryoprotected successively in 10% and 20% glycerol, frozen in a cold 2-methylbutane bath, and stored at -80°C . Finally, brains were sectioned in the coronal plane throughout their rostrocaudal extent on a sliding microtome at a nominal thickness of 40 μm , and the histological sections were kept at -80°C in storage solution (30% glycerol, 30% ethylene glycol in 0.02-M phosphate buffer; PB) until processing for in situ hybridization (RNase free conditions were maintained throughout).

2.5. Arc in situ hybridization and quantification

To allow comparisons across all behavioral conditions, 6 batches of radioactive in situ hybridization were performed, each including approximately 5 evenly spaced sections (400 μm intersection interval) from all brains examined in the study ($n = 62$). By this design, sections were approximately anatomically matched within a batch, with all behavioral conditions represented, and each batch covered a different level along the rostrocaudal axis of the brain.

The in situ hybridization procedures were adapted from Fletcher et al. (2006) and Haberman et al. (2008). All solutions were RNase free. Free-floating sections were washed twice at room temperature with 0.1-M glycine in 0.122-M PB for 3 minutes, followed by a 15-minute PB wash. Protein digestion was performed via incubation at 37°C with 1 $\mu\text{g/mL}$ proteinase K in 0.1-M Tris and 0.05-M EDTA solution (pH = 8.0) for 30 minutes. The reaction was stopped by a 10-minute wash with 0.25% acetic acid in 0.1-M triethanolamine (pH = 8.0), followed by 2 15-minute washes with $2\times$ saline-sodium citrate buffer (SSC, $1\times$: 0.15-M NaCl, 0.015-M sodium citrate). RNA probes were constructed from nearly full-length *Arc* complementary DNA (Lyford et al., 1995) inserted into a pBluescript II SK plasmid (Stratagene, La Jolla, CA, USA). Sense and antisense probes were transcribed by T3 and T7 RNA polymerase respectively, using a MEGAscript kit (Ambion, Grand Island, NY, USA). ^{35}S -UTP (Perkin Elmer, Waltham, MA, USA) was incorporated into the probe, and concentration was determined by scintillation counting. The hybridization buffer (Fisher) was supplemented with 0.25-mg/mL transfer RNA, 0.3-mg/mL sheared salmon sperm DNA, 20-mM dithiothreitol (DTT), and 1×10^7 cpm/mL ^{35}S -UTP *Arc* probe. Sections were hybridized overnight at 60°C followed by a series of posthybridization washes. Sections were washed twice for 30 minutes at 60°C in $4\times$ SSC with 0.005-M DTT, followed by a 30 minutes wash at 60°C in $2\times$ SSC with 0.005-M DTT and 50% formamide. They were then incubated with 0.02 mg/mL RNase A in 10-mM Tris, 0.5-M NaCl, and 1-mM EDTA (pH = 8.0) at 37°C for 30 minutes. Finally, the sections were washed in progressively lower concentrations of SSC (2 washes in $2\times$ SSC, 2 in $0.5\times$ SSC, and 2 in $0.1\times$ SSC). Sections were mounted onto electrostatically charged glass slides (Fisher) and allowed to dry. The slides were exposed to a phosphor screen for 7 days, which was then imaged using a Typhoon Trio+ Imaging system (GE Healthcare, Piscataway, NJ, USA) at a

resolution of 10 $\mu\text{m}/\text{pixel}$. The digitized images were used for anatomical tracing and quantification.

Based on the anatomical organization of systems implicated in place and response learning and the interaction between them, we quantified levels of *Arc* mRNA expression in the hippocampus (CA1, CA3, and dentate gyrus, DG), striatum (dorsal, DStr, and ventral, VStr), PFC (orbitofrontal, OFC; PrL; IL), and cingulate gyrus (Cg). Anatomical borders were traced and intensity values measured using ImageJ software (rsbweb.nih.gov/ij). The anterior and posterior borders were defined for each region of interest, ensuring equal sampling in the anterior-posterior axis across animals. After the anatomical parcellation of Paxinos and Watson (2007), OFC was quantified in sections from approximately Bregma 5.64 mm –4.2 mm, which yielded an average of 2.9 sections per brain. For PrL, sections from Bregma 4.68 mm –3.24 mm were analyzed, averaging 3.5 sections across animals. IL was quantified from Bregma 3.72 mm –3.24 mm, which corresponded to 1.9 sections per animal. Cg was quantified from approximately Bregma 2.04 mm to –0.36 mm, for an average of 5.0 sections per brain. DStr and VStr were quantified in sections corresponding to Bregma 2.04 mm –0.24 mm, averaging 3.0 sections per animal. Finally, the CA1, CA3, and DG subfields through the dorsal hippocampus were quantified in sections from Bregma –2.52 mm to –4.08 mm, yielding an average of 3.5 per subject. For all sections, regions of interest in both the right and left hemispheres were included in the analysis.

2.6. Statistical analysis

For the behavioral analysis, we recorded the number of trials and errors to acquire each strategy. Trials to criterion were the number of trials required to reach the target accuracy level for both the sequential and random order start arm phases of acquisition. Trials and errors were measured and analyzed for the 4 variants of place and response strategies (i.e., “go east” or “go west” and “turn left” or “turn right”). All results are presented as averages $\pm$ standard error of the mean, and significance was tested at $p < 0.05$. One AU rat displayed a unique pattern of results, dramatically different than others in the same group, illustrated most clearly by the distribution of strategy preference scores for individual subjects (see Section 3). Data for this outlier were excluded from subsequent group analyses. Repeated measures analyses of variance (ANOVAs) were performed with group (Y, AU, and AI) as the between subject variable and strategy (place1, response1, place2, and response2) as the within-subject factor. Additionally, to analyze the proportion of different error types, a nonparametric Friedman test was conducted for each group, with error type (response-like, place-like, and undetermined) as the within-subject variable.

Expression of the immediate early gene *Arc* was analyzed independently for each region of interest. The individual values for each site sampled were normalized to the average intensity observed in the region of interest in the HC group. To compare across groups, the average intensity per animal in each region of interest was used as the dependent variable in a 2-way ANOVA with age and/or cognitive group (Y, AU, and AI) and training condition (WDS, single strategy) as between-subject factors. Additionally, univariate ANOVAs were used to evaluate targeted comparisons of *Arc* values between key conditions (e.g., groups

distinguished by their success or failure on the WDS). Where appropriate, between group contrasts were computed using the least significant difference post hoc test.

All statistical analysis was conducted using SPSS PASW Statistics (version 18.0, IBM, Chicago, IL, USA) running on Mac OS X (version 10.6.6, Apple, Cupertino, CA, USA).

3. Results

3.1. Behavioral characterization

Young and aged rats were tested first on a standardized version of the Morris water maze, optimized for identifying individual differences in hippocampal integrity. Learning index scores were calculated as described in Gallagher et al. (1993) and the distribution was similar to previous studies in this model (Gallagher et al., 2003; Haberman et al., 2011; Smith et al., 2000). Because this measure reflects proximity to the escape platform across probe trials, lower scores reflect more accurate searching and better spatial learning for the escape location. For 6-month old (Y) animals that subsequently learned successive place and response strategies, the learning index scores averaged 183.8 ± 9.7 (Fig. 1, white circles, $n = 8$). Between-animal variability was greater among the aged rats (24–28 months old), and subjects were classified as AU if their scores were under the 240 cutoff (Fig. 1, gray squares, mean = 195.6 ± 7.2 , $n = 8$). AI rats scored worse than Y, above 240 (Fig. 1, black triangles, mean = 275.7 ± 8.4 , $n = 8$). Behavioral controls (e.g., rats that learned a single strategy) performed comparable to their respective experimental groups during background water maze testing. The single session of nonspatial cue conducted training at the end of the protocol revealed no differences in sensorimotor function or motivation between young and aged subjects, regardless of cognitive status.

3.2. Place and response learning

At least 2 weeks after baseline behavioral characterization, 8 Y, 8 AU, and 8 AI animals underwent T-maze training. Over the course of training, rats learned 2 place (“go East” and “go West”) and 2 response (“turn Left” and “turn Right”) strategies, alternated across days (Fig. 2A). In striking contrast to the remainder of the aged-unimpaired group (and despite performing as well as Y during background water maze training) 1 AU rat displayed substantial difficulty acquiring place strategies in the T-maze task, scoring >4 standard deviations from the mean of other AU animals for the trials to criterion measure. Results from this animal were therefore excluded from the group analyses.

3.2.1. Strategy learning—Animals initially learned a place strategy in the T-maze (either “go East” or “go West”, Place1) (Fig 3A). Young animals required an average of 6.1 ± 3.0 trials to criterion (2.9 ± 1.3 errors) totaled across the sequential and random order start arm phases of acquisition (see Section 2). The AU group required 14.4 ± 3.2 trials (9.1 ± 1.6 errors), and the AI group required 34.9 ± 6.4 trials (17.0 ± 2.5 errors). During this stage, a single strategy was enforced throughout each daily test session, and the day after mastering place1, the first response strategy was introduced (“turn Left” or “turn Right”, response1). The overall outcome was opposite the pattern for the place strategy such that the Y group now required the greatest number of trials to learn response1. Specifically, the number of

trials to criteria was 24.4 ± 5.4 for Y animals (11.9 ± 2.7 errors), 11.7 ± 3.5 trials for AU animals (5.9 ± 1.2 errors), and 8.4 ± 3.7 trials for AI animals (4.9 ± 2.3 errors). The third strategy involved learning to swim to a novel place, this time with the escape location opposite to previous testing on this task variant (e.g., “go West” for animals that started by learning “go East”, Place2). The results replicated findings for place1, with trials to criterion averaging 6.4 ± 2.1 for the Y group (2.6 ± 0.7 errors), 11.9 ± 3.3 for AU (7.0 ± 1.7 errors), and 31.8 ± 8.4 for AI rats (15.4 ± 3.4 errors). The final strategy learned was the alternate form of the previously acquired response (e.g., “turn Right” if the animals had learned “turn Left”, Response2). Similar to the pattern for response1 Y animals required 27.4 ± 6.8 trials (10.5 ± 2.3 errors), the AU group achieved the criteria in 15.7 ± 8.0 trials (7.4 ± 3.0 errors), and AI rats averaged 5.0 ± 0.9 trials to criterion (3.9 ± 1.0 errors). A repeated measures ANOVA computed for these results revealed a significant interaction between group and strategy ($F_{6,60} = 7.89$, $p < 0.001$ Greenhouse-Geisser correction for violation of sphericity) while the main effects of group ($F_{2,20} = 1.32$, $p = 0.29$) and strategy ($F_{3,60} = 0.29$, $p = 0.77$) were not statistically significant. To identify the source of the significant interaction, performance was compared across groups for each strategy. Univariate ANOVA revealed significant group effects for each of the 4 strategies considered independently (place1: $F_{2,22} = 10.78$, $p < 0.001$; response1: $F_{2,22} = 3.84$, $p < 0.05$; place2: $F_{2,22} = 6.06$, $p < 0.01$; response2: $F_{2,22} = 3.77$, $p < 0.05$), with pairwise contrasts confirming significant differences between the Y and AI groups in all cases and between AU and AI for both place strategies. Young and AU rats learned the place strategies at equivalent rates, and notably, the AU group scored similarly throughout acquisition, without changes in performance when switching between strategies. Overall, whereas Y subjects exhibited more rapid learning for place than response strategies, AI rats displayed the opposite pattern, that is, an orthogonal profile that might be predicted on the basis of differences in hippocampal integrity across these groups. The more unexpected outcome was that AU exhibited a pattern qualitatively distinct from both Y and AI, characterized by relatively rapid acquisition for both place and response.

To further characterize individual differences in the cognitive capacities assessed by the T-maze procedure, we computed a strategy preference score (see Section 2). By this metric, negative values represent relatively faster acquisition of place than response strategies, and positive values indicate the converse, with deviations from 0 reflecting the magnitude of preference. These scores (Fig. 3B) indicated that most Y animals had a clear bias favoring place strategies, while AI animals showed an equally strong or stronger preference for learning response strategies. The overall distribution for the AU group was similar to Y, with the prominent exception of a single outlier that displayed the strongest response preference of any animal tested. Performance in this animal was therefore an outlier in both quantitative and qualitative terms, justifying its exclusion from the group comparisons.

3.2.2. Error analysis—Based on the ordering of start arms and specific sequence of choices executed across trials, we were able to classify a majority of errors according to the strategy that most likely prompted incorrect responses (see Section 2.3.3 for details). The distribution of error types within groups was constant across the 2 assessments of each strategy, and results for this analysis were therefore pooled across the entire behavioral

testing procedure. As depicted in Fig. 4, most of the errors in young animals were place-like ($62.9 \pm 6.0\%$) or trials on which rats returned to a previously rewarded escape location when current task contingencies called for an opposing body turn (among the remainder, $24.7 \pm 6.8\%$ were response-like and $12.4 \pm 2.8\%$ were undetermined errors). AI animals, in contrast, exhibited an orthogonal pattern, committing a majority of errors using a response-like strategy (i.e., $61.4 \pm 3.1\%$, with $15.4 \pm 3.9\%$ place-like, and $23.2 \pm 2.3\%$ undetermined). The AU group displayed a distribution of errors intermediate between Y and AI subjects. The percentage of response-like ($45.0 \pm 8.1\%$) errors for these animals was almost twice that of the Y group but substantially lower than in the AI group, whereas the percentage of place-like errors ($31.2 \pm 8.3\%$) was higher than in AI animals and lower than in Y rats (undetermined errors represented $23.8 \pm 2.3\%$ of the total). Accordingly, unlike the strong error bias seen in both Y and AI rats, the distribution of response- and place-like errors was more nearly even in the AU group. A nonparametric Friedman test confirmed that whereas the Y and AI groups displayed a disproportionate percentage of place- and response-like errors, respectively (Y: $\chi^2 [2] = 7.75$, $p < 0.05$; AI $\chi^2 [2] = 12.97$, $p < 0.01$), errors in AU rats were evenly distributed across categories ($\chi^2 [2] = 3.92$, $p = 0.14$). Notably, the total number of errors committed during the task was not different across groups (univariate ANOVA, $F_{2,20} = 2.11$, $p = 0.15$).

3.3. Within-day switch

The WDS further emphasized cognitive flexibility and the ability to rapidly adjust behavior in response to modified task demands. In this final session, subjects were tested initially for retention of the response strategy performed the preceding day, followed by a switch to a place strategy for a maximum of 20 trials (Fig. 2B). Whereas all Y animals ($n = 8$) achieved the WDS performance criterion (averaging 3.6 ± 1.3 trials), only 3 of 8 AI rats succeeded. The 5 remaining AI animals all failed to reach the WDS criterion within the 20-trial cutoff, committing on average 9.6 ± 4.3 errors. Similar to Y, all 7 of the AU animals included in the group comparisons mastered the novel WDS (mean = 5.3 ± 2.0 trials; only the AU outlier failed the WDS criterion). Interestingly, the strategy preference scores accurately predicted WDS success. All animals with scores < 3000 , regardless of age or spatial learning capacity, were able to switch, whereas those with a strong response preference (scoring > 3000) failed. These results suggest that hippocampal integrity alone is not sufficient to account for the effects of aging on switching ability.

3.4. Behaviorally induced Arc mRNA levels

To better understand the neural network dynamics that might contribute to the different behavioral profiles we observed, a region-of-interest analysis was conducted comparing *Arc* mRNA expression across behavioral testing conditions in Y, AU, and AI animals. Rats that successfully accomplished the WDS were sacrificed 30 minutes after the first correct response in the criterion run of trials (i.e., a time point approximately 40–45 minutes after the first trial of the session). This design ensured that the relevant behavioral events occurred within the time window for *Arc* transcription and transport to dendrites but before degradation (Fletcher et al., 2014; Guzowski et al., 2001). Animals that failed to achieve the WDS criterion were sacrificed together with behavioral controls at an equivalent time point from the first trial of the day. One behavioral control group comprised animals euthanized

directly from the HC, providing a baseline of constitutive, resting *Arc* expression. A second control consisted of animals that learned only 1 strategy (either place or response), and that were sacrificed after 20 trials of testing on the current version of that strategy. These animals provided an assessment of *Arc* expression related to performance of a single task contingency, and a basis for comparison with the demands of switching between place and response strategies. Regions of interest for this analysis included the hippocampus (CA1, CA3, and DG), the striatum (dorsal, DStr and ventral, VStr), several divisions of the PFC (OFC; PrL; IL), and the cingulate cortex, (Cg).

Measured as a percentage of HC control values, behavioral testing induced significant *Arc* expression in most of the brain regions examined, including all subdivisions of the PFC, the Cg, the DStr, and the CA1 field of the hippocampus (Fig. 5). Focusing on these areas, next, we determined the influence of cognitive aging and behavioral training condition on regional network activation. *Arc* mRNA levels were equivalent in rats tested on either place or response alone (p -values > 0.5 for all regions), and these results were therefore collapsed into a single strategy control for comparison with effect of WDS training. Statistical analysis revealed reliable main effects of age and/or cognitive group for all activated regions (2-way ANOVA; OFC: $F_{5,55} = 16.5$, $p < 0.001$; PrL: $F_{5,55} = 16.82$, $p < 0.001$; IL: $F_{5,55} = 17.02$, $p < 0.001$; Cg: $F_{5,55} = 33.48$, $p < 0.001$, DStr: $F_{5,55} = 17.30$, $p < 0.001$, CA1: $F_{5,55} = 3.83$, $p < 0.05$), and post-hoc comparisons confirmed that these effects reflected the influence of chronological age, such that Y animals showed higher *Arc* levels than AU and AI. The lack of prominent differences between AU and AI rats in hippocampal *Arc* expression contrasts with earlier reports of disproportionate blunting in aged rats with spatial memory impairment (Fletcher et al., 2014; Marrone et al., 2012) and presumably relates to the very different behavioral background and induction events used across experiments.

Turning to the main effects of behavioral testing condition, the PFC was unique among the regions examined in displaying significantly greater *Arc* expression in WDS rats relative to the single strategy controls (OFC: $F_{5,55} = 7.61$, $p < 0.01$; PrL: $F_{5,55} = 8.03$, $p < 0.01$; IL: $F_{5,55} = 7.61$, $p < 0.001$). These findings are consistent with idea that the PFC is prominently engaged under conditions emphasizing cognitive flexibility and strategy selection. To explore these effects in greater detail, we directly compared *Arc* expression across the WDS and single strategy conditions within the Y, AU, and AI cohorts. The striking overall pattern was that while behavioral testing condition prominently influenced PFC *Arc* expression in both Y and AI rats, induction in AU subjects was completely insensitive to demands on strategy switching (Fig. 5A–C). Specifically, Y and AI rats displayed significantly greater *Arc* expression following WDS training than single strategy controls in all PFC divisions ($p < 0.05$ for OFC, PrL, and IL in both Y and AI), whereas levels in aged rats with normal memory (AU) were equivalent across these conditions (OFC: $p = 0.74$, PrL: $p = 0.52$, IL: $p = 0.13$). Thus, PFC activation as measured by *Arc* induction mirrored the behavioral results during task acquisition, where performance was prominently affected when Y and AI rats switched between place and response strategies, but where AU subjects learned the 2 strategies at equivalent, rapid rates (Fig. 2A).

Although main effects of behavioral testing condition were statistically significant only in the PFC, *Arc* expression in the DStr exhibited a significant interaction between group and

testing condition ($F_{5,55} = 3.85$, $p < 0.05$), likely because relative induction levels were opposite across the Y and AI groups (Fig. 5E). Whether or not this represents a reliable distinction in striatal *Arc* activation between these groups, direct contrasts revealed a significant difference between the WDS and single strategy conditions only in young rats ($p < 0.05$).

Finally, in an effort to test the possibility that regional activation patterns might contribute to age-related deficits in strategy switching, we directly compared *Arc* expression among the AU and AI rats grouped according to their success or failure in achieving the WDS performance criterion (Fig. 6). Univariate ANOVA revealed no reliable differences between these groups for most of the regions examined, including all divisions of the PFC, the cingulate, and the dorsal and ventral striatum (OFC: $p = 0.08$, PrL: $p = 0.23$, IL: $p = 0.42$, Cg: $p = 0.78$, DStr: $p = 0.91$, VStr: $p = 0.38$, CA1: $p = 0.19$, data not shown). Indeed, the hippocampus was the only area that displayed reliable effects, reaching statistical significance for both the CA3 field ($F_{1,14} = 8.35$, $p < 0.01$) and DG ($F_{1,14} = 8.47$, $p < 0.01$). As illustrated in Fig. 6, post hoc contrasts confirmed that this effect was attributable to elevated *Arc* expression selectively in AI rats that failed the WDS procedure relative to both AU and AI animals that succeeded. The number of successful AI rats available for analysis was small ($n = 3$) and it will be important to confirm this observation in larger cohorts. Nonetheless, the findings are noteworthy in suggesting that, alongside a role in spatial memory impairment (Wilson et al., 2005), excess neuronal activity in the hippocampus may be among the factors that contribute to individual differences in cognitive flexibility during aging.

4. Discussion

Studies in young subjects provide compelling evidence that the corticostriatal and hippocampal systems responsible for response and place learning, respectively, compete for the control of behavior in settings that permit both types of navigational strategies. For example, damage to the hippocampus produces profound deficits in spatial navigation but facilitates response learning in a T-maze task, whereas lesions of the dorsal striatum yield the opposite pattern (Lee et al., 2008; McDonald and White, 1993; Packard et al., 1989). The competitive interaction between memory systems presumably also underlies the normative progression of navigational strategies observed in intact subjects, where rats tested on tasks allowing both solutions initially rely on rapid place learning abilities (Tolman et al., 1946) and eventually transition to a more incremental, habit-like response approach with continued training (Chang and Gold, 2003; Packard and McGaugh, 1996). Notably, the available evidence also indicates that the functional organization of these systems is similar in laboratory animal models and humans (Iaria et al., 2003; Iglói et al., 2009; Mizumori et al., 2004; Voermans et al., 2004).

Research on the interaction between memory systems provides an illuminating window on cognitive aging. An early study in rats reported that aged subjects preferentially rely on response over place strategies for maze navigation relative to younger animals (Barnes et al., 1980), and qualitatively similar observations in older humans suggest that this is a conserved feature of aging (Bohbot et al., 2012; Rodgers et al., 2012). The current findings replicate

and extend this background, demonstrating that an age-related shift favoring response learning emerges selectively among aged rats with documented deficits in hippocampal integrity. Young adults, by comparison, exhibited a bias toward place learning. Together, the available evidence is consistent with the idea that corticostriatal control predominates in AI rats, yielding an enhanced rate of response strategy learning relative to young adults. What has not been clear from earlier studies is how aging affects the competition between memory systems under conditions that explicitly require learning for both place and response and switching between the 2. The novel observation revealed in this setting is that aged rats with preserved spatial memory exhibit a pattern of performance qualitatively different than either young adult or age-matched rats with impaired hippocampal function. Specifically, AU rats fail to display a bias for either place or response learning, acquiring both equally rapidly, without apparent disruption when switching between strategies. To the degree that performance in the Y and AI groups reflects the competitive interaction between striatal and hippocampal memory systems, the behavioral results suggest that this competition is at least partly diminished in AU rats. An alternative way of understanding the results is to suppose that performance in AU rats is mediated by a qualitatively distinct, unified cognitive schema in which the task structure comprises a continuous strategy discrimination problem, rather than the juxtaposition of conflicting strategies. Whereas this account suggests that AU rats might exhibit more rapid switching or fewer perseverative errors immediately after a change in task contingencies, targeted error analyses failed to reveal significant group differences for any of the relevant measures examined (e.g., error rate during the first 5 or 10 trials after a switch between strategies, number of perseverative errors; data not shown). Nonetheless, the notion that positive outcomes in aging can be supported by cognitive adaption merits continued attention in animal models amenable to mechanistic investigation.

Expression of the plasticity-related immediate-early gene *Arc* has proved to be a powerful tool for mapping neural network activation patterns induced by behavioral training and associated cognitive demands (Guzowski et al., 1999). Studies using this approach have reported multiple effects of aging, including blunted expression in the hippocampus after open field exploration (Marrone et al., 2012) and a failure of both mRNA and protein induction specifically in aged rats with spatial memory deficits (Fletcher et al., 2014). The pattern in the present experiments was somewhat broader, consisting of an overall age-related decline in *Arc* expression in all regions that displayed behavioral induction, independent of cognitive status (Fig. 5). A potential account of these differences across experiments is that, in the present study, rats received extensive training over many daily test sessions in the same environment, in contrast to previous research where *Arc* induction was examined after exposure to a relatively novel setting (Fletcher et al., 2014; Marrone et al., 2012). Investigations examining the effects of fornix transection demonstrate that *Arc* expression is specifically coupled with plasticity-related activity, and that neuronal firing alone is not sufficient for induction (Fletcher et al., 2006; Miyashita et al., 2009). On the basis of these findings, combined with evidence that certain forms of overtraining can blunt *Arc* expression (Guzowski et al., 2006; Kelly and Deadwyler, 2002), it seems reasonable to suspect that extended testing is an important factor contributing to the pattern of behavioral induction reported here, including the generalized effect of chronological aging.

Alongside a baseline of overall age-related decline, group differences in *Arc* expression as a function of demands on cognitive flexibility and strategy switching were seen exclusively in the PFC. A potential mechanistic basis is suggested by recent data pointing to an increase in tonic PFC inhibition in aged rats with working memory impairment (Banuelos et al., 2014), mediated at least in part by altered GABAergic signaling (McQuail et al., 2015). In young rats, inactivation of the PrL and IL divisions of the PFC cause impairments on tasks that require switching between place and response-based navigational strategies, at least early in the course of acquisition (Ragozzino et al., 1999; Rich and Shapiro, 2007), while OFC damage leads to deficits on intradimensional strategy reversals (McAlonan and Brown, 2003). Corresponding electrophysiological findings demonstrate that neuronal firing in the PrL and IL is prominently modulated when rats switch between place and response strategies, relative to when animals engage in the same behavior in the context of performing a single strategy (Rich and Shapiro, 2009). A recent study also showed that activity in PrL can represent the navigational rule implemented by animals on a trial to trial basis (Powell and Redish, 2014).

Consistent with the idea that demands on cognitive flexibility engage the PFC, here we found elevated *Arc* expression in PrL, IL, and OFC in young animals tested on the WDS procedure shortly before sacrifice, compared with rats that received a comparable duration of training on a single place or response strategy. A qualitatively similar pattern was seen in aged rats with memory impairment, despite the inability of most AI rats to accomplish the WDS. These findings suggest that the blunted *Arc* activation found in the aged PFC is nonetheless increased when switching is required against a background of marked imbalance between place and response learning systems, as a consequence of hippocampal dysfunction. This balance between memory systems is presumably normalized in aged rats with preserved hippocampal integrity, and in this setting the age-dependent reduction in tonic PFC drive may relieve place and response systems from top-down control. Consistent with this proposal, AU rats, unlike either Y or AI subjects, learned place and response strategies with equal facility and transitioned between the 2 without disruption. A plausible alternative, however, is that differences in prefrontal *Arc* activation might reflect, rather than determine strategy switching abilities. Whichever interpretation is confirmed in future research, our findings add to a growing body of evidence that successful outcomes in aging reflect a unique condition, qualitatively different than young with respect to both cognitive function and underlying neurobiology (Haberman et al., 2011). Findings of this sort have prompted interest in the idea that, alongside efforts to understand the basis of decline, efforts might profitably focus on identifying the neuroadaptive mechanisms that mediate positive aging trajectories.

Neurobiological markers of poor cognitive outcome in both the model studied here (Wilson et al., 2005), and humans (Yassa et al., 2011), include elevated neuronal activity in the DG and CA3 region of the hippocampus. It is interesting in this context that, among the areas examined, the CA3/DG region was unique in displaying a pattern of *Arc* expression that distinguished between aged rats that succeeded or failed to master the WDS procedure. The reliability of these observations requires confirmation in larger numbers of subjects and they may relate to variables associated with the number of unsuccessful escape attempts, such as stress. Nonetheless, the findings are consistent with the possibility that the cognitive

consequences of hippocampal hyperactivity extend beyond spatial memory, contributing to the effects of aging on the interaction between memory systems. Pharmacological treatments that normalize excess activity in the aged hippocampus improve spatial memory (Bakker et al., 2012; Koh et al., 2010), and testing whether the same treatment rescues cognitive flexibility is an important direction for investigation.

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Fig. 1.

Learning index scores for individual animals in a standardized Morris water maze task. Higher scores indicate poorer performance (see Gallagher et al., 1993). Aged animals with scores comparable to young (Y; white circles, $n = 8$) were classified as unimpaired (AU; gray squares, $n = 8$), and those with scores outside the Y distribution were classified as impaired (AI; black triangles, $n = 8$). *Learning index for the aged rat with outlier performance on the strategy switching task. Abbreviations: AI, aged impaired; AU, aged unimpaired; Y, young.

Fig. 2. Schematic of behavioral training for (A) acquisition of response and place strategies and (B) within-day strategy switching (see text for details).

Fig. 3.

(A) Mean trials to criterion ($\pm$ standard error of the mean) for successively acquired place and response strategies in Y ($n = 8$), AU ($n = 7$), and AI rats ($n = 8$). (B) Strategy preference scores ($[(\text{trials to criterion for place strategies} - \text{trials to criterion for response strategies}) \times \text{total trials to criterion}]$) for individual subjects. *Score for an AU outlier that was excluded from the group comparisons. Abbreviations: AI, aged impaired; AU, aged unimpaired; Y, young.

Fig. 4. Percent distribution of error types for (A) young, (B) aged unimpaired, and (C) aged impaired rats during acquisition of successively learned place and response strategies.

Fig. 5.

Mean percent Arc messenger RNA expression (±standard error of the mean) relative to home cage (HC) controls in rats tested on the within-day switch or a single strategy; (A) orbitofrontal cortex (OFC), (B) prelimbic prefrontal cortex (PrL), (C) infralimbic prefrontal cortex (IL), (D) cingulate cortex (Cg), (E) dorsal striatum (DStr), (F) CA1 region of the hippocampus. The bottom row shows brain regions in which no significant behavioral induction was observed: (G) ventral striatum (VStr), (H) CA3 region of the hippocampus, and (I) dentate gyrus of the hippocampus (DG). Panel inserts illustrate the regions

quantified. [#]Two-way analysis of variance (ANOVA) main effect of cognitive group, $p < 0.05$; [†]2-way ANOVA main effect of behavior, $p < 0.01$; [§]ANOVA cognitive group by behavior interaction, $p < 0.05$; *Student t test, $p < 0.05$. Abbreviations: AI, aged impaired; AU, aged unimpaired; WDS, within-day switch; Y, young.

Fig. 6.

Mean percent *Arc* mRNA expression ($\pm$ standard error of the mean) relative to home cage (HC) controls in aged rats grouped according to their success or failure during testing on the within-day switch. Regions outside the hippocampus failed to reveal any group differences.

#Univariate analysis of variance, $p < 0.01$.