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Effects of ApoE genotype and mild cognitive impairment on implicit learning

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Abstract

The goals were to investigate implicit learning in mild cognitive impairment (MCI), and to determine the relations of implicit learning systems to apolipoprotein E (ApoE) genotype in healthy controls. Elderly controls grouped by ApoE status (ApoE-e4 allele carriers versus ApoE-e4 allele non-carriers) and MCI patients participated in the study. Individuals in all three groups completed both contextual cueing and serial reaction time (SRT) tasks. In the former, people learn to use repeated spatial configurations to facilitate search for a target, whereas in the latter, they learn to use subtle sequence regularities to respond more quickly and accurately to a series of events. Results revealed that healthy elderly individuals carrying the ApoE-e4 allele showed contextual cueing deficits compared to those who did not carry the ApoE-e4 allele. Further, elderly controls carrying the ApoE-e4 allele revealed similar amounts of contextual cueing as the MCI group, while the non-carriers performed better. Sequence learning, by contrast, was uninfluenced by either MCI or by ApoE genotype in healthy controls. This study provides further support for the medial temporal lobe dysfunction and relative integrity of fronto-striatal systems in MCI, and indicates the influence of ApoE genotype on implicit learning even in healthy older individuals without cognitive impairment.

Keywords: Implicit learning; Mild cognitive impairment; ApoE genotype; Spatial contexts; Sequence learning; Aging

1. Introduction

Individuals who ultimately develop a degenerative dementia such as Alzheimer's disease (AD) likely transition through a period of mild impairment. Mild cognitive impairment (MCI) is a term used to describe this transitional zone between normal aging and very early dementia. Mild cognitive impairment represents a condition where individuals show memory impairment greater than expected for their age, but otherwise are functioning well and do not meet the criteria for dementia [33]. As such, the concept of MCI has generated a great deal of interest from both clinical and research perspectives.

It is established that explicit forms of learning and memory, such as delayed recall and list learning, decline in MCI [13,33]. The picture is less clear, however, for implicit learning, which has been studied relatively little, and which has multiple forms, each calling upon distinct neural substrates. Implicit learning generally refers to a situation where a person learns about the structure of a stimulus environment without conscious effort to learn and without ability to describe what has been learned [34]. Studies of implicit learning in AD have shown a relatively preserved implicit learning system in patients with very mild AD [11,21], whereas those in the mild stage revealed impairments [8].

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Whether implicit learning is preserved in MCI, however, and which forms, is unknown since, to date, no studies have been reported on implicit learning in MCI. Addressing this question is important because implicit learning, with its multiple forms, could be useful in the dissociation of MCI from normal aging. That is, if some forms of implicit learning are spared in MCI while others are not, this pattern could help differentiate MCI from normal aging, thereby aiding in early diagnosis of MCI. A relatively intact implicit learning system might also be useful in designing programs that increase the period during which patients can be independent.

The extent to which implicit learning is influenced by ApoE status is also unknown. ApoE genotype is a well-documented risk factor for development of AD [5,37], and recently, it has been shown as a strong predictor of progression to AD in MCI patients [32]. AD patients carrying the ApoE-e4 allele have also shown pronounced medial temporal lobe atrophy [18,24]. Further, the ApoE gene has been linked to several cognitive processes, such as spatial attention [30] and working memory [12], where ApoE-e4 carriers have shown deficits compared to ApoE-e4 non-carriers. Currently there are no published data on the relation between implicit learning and ApoE genotype. We are aware of only one previous study on the relation between genotype and implicit learning [20]. Keri et al. investigated the relationship between dopamine D_3 receptor (DRD3) genotype and learning on a weather prediction task, a probabilistic classification task involving implicit processes, particularly in the early phase [23]. They found that early, but not late, learning was associated with DRD3 genotype, thereby establishing that one form of implicit learning is influenced by genotype.

We investigated effects of ApoE genotype and MCI on implicit learning using two different paradigms—sequence learning and contextual cueing. This is of particular interest because the two tasks call upon different neural systems that are known to be differentially affected in MCI; hence, they provide insights into the differential neuroanatomical and cognitive changes associated with MCI, as well as the relations of implicit learning systems to ApoE genotype.

To investigate sequence learning, we used the serial reaction time (SRT) task developed originally by Nissen and Bullemer [27]. In this task, participants respond to a visual stimulus that appears in one of several spatial locations by pressing the corresponding key as quickly as possible. Unbeknownst to participants, however, stimuli follow a predetermined repeating sequence. Participants typically encounter several blocks containing the predetermined pattern, followed by a random block where the pattern is removed. Implicit learning is inferred from the difference in performance (on speed and accuracy) between pattern and random blocks, without ability to develop awareness about the regularity. Several studies of brain injured patients as well as neuroimaging studies of healthy adults indicate that such learning of sequences is mediated by the fronto-striatal-cerebellar system [6,9,15,38]. For example, studies of Parkinson's and Huntington's disease patients with damage to the basal ganglia, as well as cerebellar patients have shown sequence-specific learning deficits on the SRT task, indicating the involvement of the striatal dopaminergic system and cerebellum in sequence learning [7,10,14,22]. Neuroimaging studies have also shown the involvement of fronto-striatal regions during sequence learning [36,38].

The contextual cueing paradigm, on the other hand, is a visual search task developed by Chun and colleagues to study how spatial context is learned [3]. In this task, people are asked to search for a target (e.g., a horizontal T) in an array of distractors (rotated L's). Unbeknownst to participants, some displays contain repeated configurations that provide a contextual cue to the location of the target, while novel displays are generated randomly. Results reveal that with practice, people respond faster to repeated than to new configurations [1,29]. Furthermore, such learning has been shown to occur implicitly in that people do not develop explicit knowledge of the relationship between the spatial context and the target location. Studies of brain-injured patients indicate that such contextual learning depends on the medial temporal lobe structures, such that amnesic patients with damage to this region show contextual cueing deficits [4,25]. Further, a dissociation has also been shown in healthy aging such that sequence learning, which depended on the age-susceptible fronto-striatal system, was impaired in healthy older adults compared to young people, whereas contextual cueing, which relied on the less vulnerable medial temporal system, was relatively preserved [16].

We administered both of the above paradigms to three groups: MCI patients, healthy elderly controls who carry the ApoE-e4 allele (Control Carriers), and healthy elderly controls who do not carry the e4 allele (Control Non-Carriers). We hypothesized that on contextual cueing, which relies on the integrity of medial temporal lobe system, healthy elderly carriers would show similar amounts of cueing as the MCI group, while the healthy non-carriers would perform better than the other two groups. In contrast, we predicted that the SRT task, which depends on the fronto-striatal system, would not be influenced by MCI status or ApoE genotype.

2. Methods

There were 24 MCI patients and 24 healthy elderly controls, the latter grouped by ApoE genotype (11 Control Carriers and 13 Control Non-Carriers). Control Carriers were those with genotypes E2/E4, E3/E4, or E4/E4, whereas Control Non-Carriers were those with genotypes E2/E2, E2/E3, or E3/E3. ApoE status was not divulged to participants. The ApoE data for the MCI group are not reported because these data were not available on all participants.[1] The mean age

[1] The ApoE data were available for 19 out 24 MCI patients, where 9 were ApoE-e4 carriers and 10 were non-carriers. Implicit learning data by ApoE status are not reported for this group because of missing data and also because, by chance, the order in which the implicit learning tasks were

and education, respectively, for the three groups were as follows: 78.5 (S.D. = 5.2) and 13.6 (S.D. = 2.7) for Control Carriers, 74.5 (S.D. = 4.5) and 13.8 (S.D. = 2.4) for Control Non-Carriers, and 77.1 (S.D. = 6.0) and 13.8 (S.D. = 3.2) for MCI patients.

Participants were recruited through the Mayo Alzheimer's Disease Patient Registry (ADPR) at the Mayo Clinic, Rochester, MN. Individuals participating in the ADPR undergo approximately annual clinical evaluations, brain MRI, neuropsychological evaluation, and basic laboratory tests. Diagnoses are established via a consensus meeting of behavioral neurologists, neuropsychologists, geriatricians, neuropsychiatrists, and nurses. The diagnosis of MCI was made in accordance with criteria established in Petersen et al. [33]. Controls were individuals who: (1) are independently functioning community dwellers, (2) do not have active neurological or psychiatric conditions, (3) have no cognitive complaints, (4) have a normal neurological exam, (5) are not taking any psychoactive medications in doses that would impact cognition [17]. Additional informed consent was obtained to recruit the participants in the present study. The study was approved by the Mayo Institutional Review Board. Subjects received $25 for participation.

2.1. Apparatus and behavioral paradigms

2.1.1. SRT task

Participants were seated in front of a Macintosh G4 computer with a 15-in. monitor (Apple Computer, Inc., Cupertino, CA). The computer displayed four open circles (0.5° each) arranged horizontally on the screen. On each trial, a target, one of the circles filling in with a black color, appeared on the screen. Participants were instructed to rest the index and middle fingers of each hand on the "z", "x", ".", and "/" keys (marked with orange stickers) and respond to the target by pressing the corresponding key as quickly as possible. The left-most position corresponded to the "z" key, while the right-most position corresponded to the "/" key. The circle remained filled in until participants pressed the correct key, at which time it disappeared and another target appeared after a delay of 120 ms.

Unbeknownst to participants, the order in which the circles filled followed a predetermined pattern. The pattern contained an 8-item sequence, which was 1-3-4-1-2-4-3-2 for half of the participants in each group, and the remaining half received the reverse pattern, 2-3-4-2-1-4-3-1. Participants were given four blocks of the sequence, followed by a fifth block in which the given pattern was reversed, and then a final pattern block (i.e., P-P-P-P-R-P, where P stands for pattern and R stands for reverse). Each block began with 4 random trials (for warm-up) and was followed by 80 experimental trials. For pattern blocks, the 8-item sequence was repeated 10 times, whereas in the fifth reverse block, the reverse of the sequence was repeated 10 times. We chose the reverse sequence for the fifth block, instead of random, to ensure that pattern and reverse blocks contained an equivalent level of sequence structure, and that any differences between the two blocks are due at least to learning of the specific pair-wise contingencies in the pattern relative to the reverse block. That is, the pattern and reverse sequences are identical in that both contain an equal number of each element and neither contains repetitions. Thus, the simplest regularity to be learned concerns pair-wise regularities; in particular, that the pairs 31, 14, 42, and 23 occur in one of the sequences, whereas 13, 41, 24, and 32 occur in the other. The other four pairs occur equally often in both forward and reverse sequences.

2.1.2. Contextual cueing task

In this task, participants were asked to locate and identify a target item among 11 distractors shown as white characters on a gray background. The target was a horizontal T with the tail pointing either left or right, and the distractors were *L*s randomly rotated by 0°, 90°, 180°, or 270°, as used in Chun and Phelps [4]. Each element subtended approximately 1.1° of visual angle at a viewing distance of 56 cm. Arrays were generated by randomly placing the 12 items into cells of an invisible 6 × 8 (rows × columns) grid. Across arrays, target location was balanced for eccentricity with respect to the center of the screen as well as for left and right screen half. Targets never appeared in the four center cells or at the extreme corners of the display grid. Every element was randomly repositioned within its cell by ±2 pixels along each axis to avoid colinearity with other elements. A set of 12 arrays was constructed for repeated presentation (details are given below). Individuals within each group received a different set of new and repeated configurations, but the same sets were used across groups with their presentation order randomized.

2.2. Procedure

Each participant completed both the contextual cueing and the SRT tasks, with the order of the tasks counterbalanced within each group. For the SRT task, they were seated in front of the computer and were given the following instructions: "In this study, we are trying to learn more about how

administered was confounded with ApoE status for the MCI group, but not for the healthy controls. That is, as described below, we counterbalanced the order of SRT and contextual cueing tasks across the MCI and controls groups, such that half of the participants in each group received SRT first (contextual cueing second) while the remaining half received contextual cueing first (SRT second). This led to 2 of the 10 non-carrier MCI patients receiving SRT first, while the remaining 8 received contextual cueing first. Of the nine ApoE-e4 carrier MCI patients, six received SRT first, while the remaining three received contextual cueing first. Thus, any effects of task order and ApoE status could not be separated for the MCI patients for whom we had ApoE data. In contrast, task order and ApoE status were not confounded for the controls; 6 of the 13 Control Non-Carriers received SRT first, while the remaining 7 received contextual cueing first. Of the 11 Control Carriers, 6 received SRT first, while the remaining 5 received contextual cueing first.

practice affects motor performance. We want to find out just how much people are able to speed their responses when they are given extended practice on a simple reaction time task". They were not given any information about the regularity that was embedded in this task. Participants completed six blocks of this task as described above. At the end of each block, the computer displayed an end-of-block feedback, which gave participants their speed information on the most recent block and the immediately preceding block. In order to minimize fatigue, participants were asked to rest their eyes for at least 30 s in between blocks, and to take additional breaks as needed.

Next, participants were given a recognition task followed by a post-experimental interview in order to assess their knowledge of the pattern embedded in the task. For the recognition task, participants were shown 10 randomly ordered trials, 5 containing the pattern sequence, and 5 containing the reverse sequence. Each trial consisted of 16 stimuli where the 8-item sequence (for pattern trials) and reverse of the sequence (for reverse trials) was repeated twice. Each stimulus was presented for 500 ms. The pattern trials contained the same sequence arrangement as the ones participants encountered during their SRT session. At the end of each trial, the computer displayed the following:

"Did this sequence occur before?"

(certain it did not) 1. . .2. . .3. . .4 (certain it did)

Their task was to observe the sequence closely and indicate their certainty of its occurrence in the SRT session on the scale of 1–4 as shown above.

The post-experimental interview assessed whether participants had gained any verbalizable pattern knowledge. The experimenter read aloud the following questions one at a time and recorded participants' responses. (1) Did you notice anything to report regarding the task? (2) Did you notice anything special about the task or the materials? (3) Did you notice any regularity in the way the stimulus was moving on the screen? If subjects answered, "yes" to question 3, they were asked (4) Did you attempt to take advantage of any regularities you noticed in order to anticipate subsequent targets? If so, did this help? (5) In fact, there was some regularity to the sequences you observed. What do you think it was? That is, try to describe any regularity you think might have been there.

For the contextual cueing task, participants were told to "locate the 'T' on the screen, determine which way it is facing and press the key that corresponds to that direction as QUICKLY and as ACCURATELY as possible". They began by completing a 24-trial practice block. Trials began with a white fixation dot approximately 0.5° centered on the screen. After 1 s the dot was replaced by a search array and the participant was to press a key indicating the target orientation ("z" for left and "/" for right pointing). They were informed that "an occasional error is acceptable (e.g., 1 error per block of 24 trials)". Auditory feedback was provided after every response (a beep or tone to signal correct or error responses, respectively). A different search array was presented on each trial in the practice block. Participants then completed 20 learning blocks of 24 trials each. Learning was similar to practice except that only 12 of the search arrays were new in each learning block (new configurations). The remaining 12 arrays (repeated configurations) were repeated across blocks, appearing once in each block. The repeated configurations predicted the location of the target element, but not its orientation. Presentation order was randomized within blocks, and people were encouraged to take a short break between blocks.

After the final learning block, people were asked a series of questions to obtain insights into their strategy and their declarative knowledge of the task. The first three questions were open-ended: (a) "Do you have anything to report regarding the task?" (b) Did you notice anything special about the task or the material?" (c) "Did you notice anything special about the way in which the stimuli were presented? If so, please explain". The last three questions asked specifically about repetitions: (d) "Did you notice whether certain configurations (spatial layout or locations of the items) were being repeated from block to block?" (e) "If so, when did you begin to notice this repetition?" (f) "Did you explicitly try to memorize any of the configurations?"

Next, people were given a single 24-trial recognition block, consisting of the 12 repeated configurations and 12 others not presented during learning, in random order. On each trial people judged whether they had seen "a display with items in the same screen positions as this earlier in the experiment". They responded by pressing either a key labeled 'yes' or one labeled 'no'. They were urged to guess if they were unsure. No feedback was provided. The whole procedure lasted approximately 2 h.

3. Results

3.1. Data reduction and statistical analysis

For the SRT task, the median RT for correct trials was calculated separately for each block, and the median RT on each of the blocks was determined for every participant. For the contextual cueing task, the 20 blocks were grouped into five epochs, each containing four blocks. For each participant, a mean response time (RT) was determined separately for correct responses to new and repeated configurations on each block. The mean RTs were then averaged across blocks to obtain a mean RT for each individual and configuration type (new or repeated) on each epoch. The main form of analysis was mixed design ANOVAs, with simple effects analyses and post hoc comparisons carried out as appropriate. An alpha level of 0.05 was used throughout, with results meeting the 0.10 level being reported as marginal. Significance tests were always two-tailed.

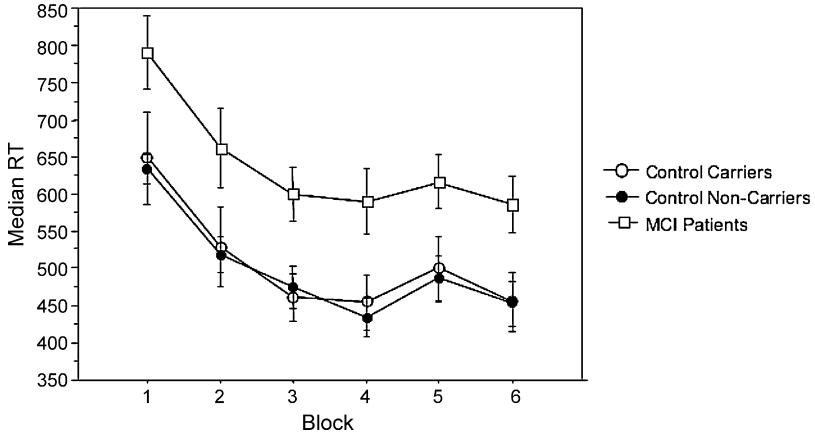

Fig. 1. Performance on response time measure across blocks for Control Carriers (open circle), Control Non-Carriers (filled-in circle), and MCI patients (open square). Error bars represent one standard error for each plotted point.

A similar data reduction was performed on accuracy. As is typical with these tasks, accuracy levels were high. On the SRT task, the overall accuracy for the MCI group was 92% and that of the control groups was 95%; on the contextual cueing, they were 96% and 97% for the MCI and control groups, respectively. When analyses on accuracy parallel to those on RT below were carried out, the findings were in a direction consistent with the RT measure, though usually not significant due to the ceiling effects. Hence, in the interest of space, we only report data from the RT measure.

3.2. SRT learning

Fig. 1 shows response times across blocks for the three groups. As the figure indicates, first, each group showed an overall skill learning effect, as evidenced by the decrease in response times across the first 4 blocks, and second, MCI patients were the slowest of the groups. In keeping with these observations, a Group (Control Carriers versus Control Non-Carriers versus MCI) × Block (1–4) ANOVA performed to determine the overall skill learning effect revealed significant main effects of Block, $F(3, 135) = 95.73$, $MS_E = 3437.04$, and Group, $F(2, 44) = 3.71$, $MS_E = 124549.85$. The Group × Block interaction was not significant ($p > 0.10$).

Pattern learning in this task is measured by subtracting the RT on the final two pattern blocks (average of blocks 4 and 6) from that on the reverse block (block 5), and the mean of these learning scores for each group are shown in Fig. 2. Here again, all three groups revealed pattern sensitivity such that performance was disrupted in the fifth block that contained the reverse sequence. In keeping with these observations, the Group × Block Type (Pattern Blocks 4 and 6 versus Reverse Block 5) ANOVA revealed a main effect of Block Type, $F(1, 45) = 25.72$, $MS_E = 1255.26$, and the Group × Block Type interaction was not significant, $F(2, 45) = 0.599$, $MS_E = 1255.26$. Further, separate ANOVAs on each group showed main effects of Block Type for all three groups suggesting that each group had learned the sequence; $F(1, 23) = 4.92$, $MS_E = 1933.51$, for MCI, $F(1, 10) = 19.76$, $MS_E = 573.50$ for Control Carriers, and $F(1, 12) = 22.59$, $MS_E = 523.44$ for Control Non-Carriers. In addition, separate ANOVAs on the learning scores comparing each pair of groups revealed no main effects of group, suggesting that there were no group differences in sequence learning ($p > 0.10$).

3.3. Contextual learning

Fig. 3 shows the mean response times of new and repeated configurations across epochs for the three groups. The figure indicates that, as had been the case for the SRT task, MCI patients were the slowest of the groups and all three groups exhibited non-specific skill learning on contextual cueing. The main effects of Group, $F(2, 45) = 3.20$, $MS_E = 2.19$ and of Epoch, $F(2, 180) = 6.14$, $MS_E = 0.036$, were significant. The Group × Epoch interaction did not reach significance ($p > 0.10$) suggesting similar skill learning for all three groups. There was also a significant main effect of

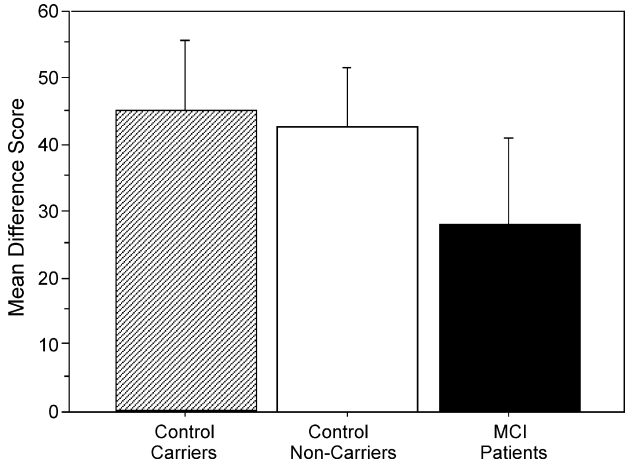

Fig. 2. Learning scores (RT on Block 5 minus mean RT on Blocks 4 and 6) on SRT task for Control Carriers, Control Non-Carriers, and MCI patients. Error bars represent one standard error.

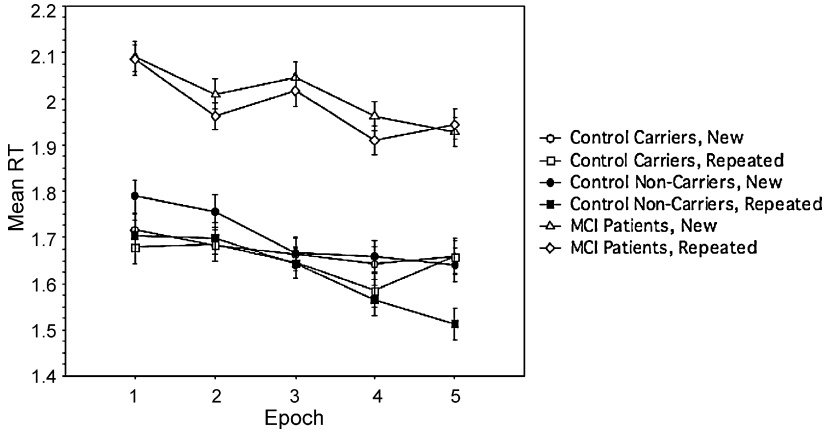

Fig. 3. Performance on response time measures across epochs for Control Carriers New (open circle), Control Carriers Repeated (open square), Control Non-Carriers New (filled-in circle), Control Non-Carriers Repeated (filled-in square), and the MCI patients New (open triangle), MCI patients Repeated (open diamond). Error bars represent one standard error for each plotted point.

Configuration, $F(2, 45) = 7.73$, $MS_E = 0.030$. The figure suggests that the Control Non-Carriers have a larger and more consistent epoch effect than the other two groups, but the configuration by group interaction did not reach significance ($p > 0.10$). Nonetheless, separate ANOVAs on each group suggested that there might be group differences in contextual cueing in that the Control Non-Carriers yielded a significant main effect of Configuration $F(1, 12) = 5.56$, $MS_E = 0.040$ but the other two groups did not; $F(1, 10) = 1.17$, $MS_E = 0.013$ for the Control Carriers, and $F(1, 23) = 2.10$, $MS_E = 0.032$ for the MCI group.

To provide a more sensitive measure of group differences in context learning, which would be comparable to the sequence learning score, we calculated a learning score from the last epoch (RT on new configurations minus repeated configurations in Epoch 5) for Control Carriers, Control Non-Carriers and MCI groups. As Fig. 4 indicates, and unlike the SRT task, group differences were observed in the contextual learning. The Group (Control Carriers versus Control Non-Carriers versus MCI) × Configuration (New versus Repeated) ANOVA on these data from Epoch 5 revealed a significant Group × Configuration interaction, $F(2, 45) = 3.34$, $MS_E = 0.016$. Separate ANOVAs on each group showed a main effect of Configuration for Control Non-Carriers, $F(1, 12) = 9.08$, $MS_E = 0.014$ but not for Control Carriers, $F(1, 10) = 0.009$, $MS_E = 0.014$ or the MCI group, $F(1, 23) = 0.138$, $MS_E = 0.019$. Further, unpaired t-tests comparing each pair of groups revealed significant group differences between Control Carriers and Non-Carriers, $t(22) = -2.12$, as well as between Control Non-Carrier and MCI groups, $t(35) = 2.43$. The Control Carrier and MCI groups, however, did not differ from each other, $t(33) = 0.148$. These findings implicate the sensitivity of the contextual cueing paradigm to ApoE genotype, in that it revealed group differences even in elderly individuals without cognitive impairment. In fact, the amount of contextual learning was related, not to MCI status, but to ApoE genotype, with the only group showing significant contextual learning being Control Non-Carriers.

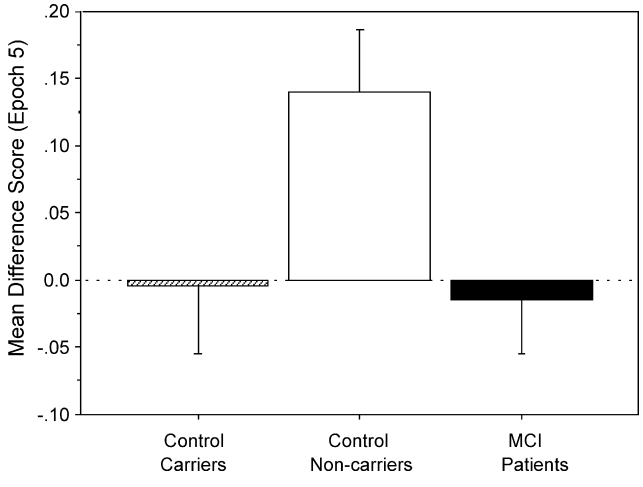

Fig. 4. Learning scores (RT on new configurations minus repeated configurations in Epoch 5) on contextual cueing task for Control Carriers, Control Non-Carriers, and MCI patients. Error bars represent one standard error.

3.4. Was the learning implicit?

3.4.1. Verbal reports

Post-experimental interviews at the end of each paradigm revealed participants did not gain awareness about the regularities embedded in either of the tasks. Many people (34 out of 48, 71%) reported that they thought there must have been some kind of pattern even though they couldn't describe it; while others said that they did not notice any regularity. Participants reported that they primarily focused on responding to targets as quickly as they could, and as such, were unable to gain awareness about the embedded regularities. For instance, one participant, when asked if she noticed any regularity in the SRT task, reported, "I didn't pay attention to that; I was too busy pressing keys", while another patient said, "no, I just automatically did it." When asked if they could make guesses as to what the pattern may be, almost half of the participants

(48%) responded saying, "I don't know." Those who made guesses usually did so on the SRT task, and there, while a few people were able to describe some parts of the sequence (such as for example, that it went in succession, which it did for some participants), no one was able to explicitly recognize the sequence or knew its length.

Although they were not able to describe the regularities, people did seem to realize that they were learning something and that it was influencing their performance. For instance, 42% reported that they got into a "rhythm" or that they anticipated where the next target would occur, especially in the SRT task. One participant said, "I wondered if I hit the key to make it appear where it did; it is almost like if you get a rhythm you can do it before the dots appeared". People also reported, however, that their anticipatory responses didn't always help them, but instead "threw them off" sometimes. One patient described it saying, "my fingers don't know what my head is thinking".

Thus, verbal reports revealed that the majority believed that some regularity was present and that it was influencing their performance in ways they could not control, but the learning is implicit in that participants were not able to describe the regularities.

3.4.2. Recognition tests

In addition to verbal reports, we examined the Recognition tests given at the end of each task to determine the extent to which participants were able to discriminate between frequently occurring and infrequently occurring events in each of the tasks. Results revealed that, on the SRT task, neither group was able to differentiate between Consistent and Backward sequences in their confidence ratings (Control Carriers: mean difference = 0.091, S.E. = 0.12, Control Non-Carriers: mean difference = 0.077, S.E. = 0.07, and MCI: mean difference = 0.092, S.E. = 0.08). The Sequence × Group ANOVA did not show any effects approaching significance, and subsequent ANOVAs on each group failed to reveal a Sequence effect ($p > 0.10$). Likewise, the contextual cueing paradigm revealed that neither group gave significantly higher confidence ratings to repeated than new configurations (Control Carriers: mean difference = 0.008, S.E. = 0.07, Control Non-Carriers: mean difference = 0.013, S.E. = 0.14, MCI: mean difference = 0.073, S.E. = 0.12). Subsequent ANOVAs were also non-significant ($p > 0.10$).

4. Discussion

We set out to investigate implicit learning of sequential regularities and spatial contexts in MCI, and to determine the extent to which these two forms are influenced by ApoE genotype. We found, first, that learning in both tasks occurred implicitly in that people were unable to either describe what they had learned in verbal reports or to distinguish between frequently occurring and infrequently occurring events on the recognition tests. We also observed that healthy elderly controls carrying the ApoE-e4 allele showed contextual cueing deficits compared to those who did not carry the ApoE-e4 allele, while by contrast, sequence learning appeared not to be influenced by ApoE genotype. To our knowledge, this is the first study to show that implicit learning of spatial contexts could be influenced by ApoE genotype, even in older adults without a cognitive impairment. It is also interesting to note that Control Carriers revealed similar amounts of contextual learning as the MCI group, while the non-carriers performed better. While this suggests that the contextual cueing paradigm might predict conversion to MCI, the present study does not demonstrate this directly, and longitudinal studies are required to address this question. It appears, then, that the contextual cueing paradigm is sensitive not only to MCI status, but also to ApoE genotype in that it could detect group differences based on the presence or absence of ApoE-e4 allele even in individuals who do not have cognitive impairment.

The implicit learning paradigms employed in this study revealed important differences between MCI and healthy controls, in that learning of contextual cues, which relies on the integrity of the medial temporal lobe system, was differentially affected in MCI, while sequence learning, which depends on the fronto-striatal system, remained intact. The SRT paradigm employed in the present study adds to earlier SRT research in AD in that it contained regularities that are more subtle than those used in previous SRT studies [8,21]. That is, unlike the original SRT task where people can learn zero-order information (i.e., the relative frequency of individual events), in the present task the lowest level of regularity to be learned is 1st order (i.e., which pairs of events are more likely to occur). People could not have learned item-information because every item (and some pairs of items) occurred equally often both in pattern and reverse blocks. Thus, the finding that learning of such a subtle regularity was unimpaired in MCI, and unaffected by ApoE genotype, provides an even stronger case for the extent to which this learning system is preserved in MCI.

Our finding that MCI patients showed deficits in contextual learning appears to be contrary to previous research that has established prominent deficits in explicit, but not implicit, systems in diseases involving medial temporal lobe dysfunction, such as AD and amnesia. The present study, however, as well as several others in recent years, call into question the broad explicit/implicit distinction and the notion of multiple memory systems based on conscious accessibility. The implicit/explicit distinction is well demonstrated in many different studies, including those involving brain injured patients, normal aging, as well as neuroimaging of young adults [6,7,9,10,14,16,36,38]. For example, explicit learning, but not implicit, is impaired in amnesic patients [28], whereas patients with Parkinson's or Huntington disease show impairment in implicit learning even though their explicit learning system remains intact [10,19]. As such, the notion of multiple memory systems based on consciousness serves as one of the most useful frameworks in learning and

memory research. Nonetheless, this notion has some limitations, including contamination of one system by the other, and difficulty in characterizing conscious learning, especially in animal model and computational studies. Consequently, an alternative conceptualization that has recently emerged is based on the type of processing involved, i.e., whether a task requires item processing or an associative processing, which involves binding of cues into a cohesive unit [2,26,35]. According to this view, it is the associative processing required in a task, regardless of whether the task is explicit or implicit, which leads to the impairments observed in amnesic and AD patients, because such learning requires the integrity of the medial temporal lobe system. Thus, to the extent that implicit tasks also require such relational learning of events, there will be deficits in patients with medial temporal lobe dysfunction, because such tasks, although implicit, still require binding of cues and context. This conceptualization gets its credence from several studies in recent years.

Park et al. observed impairments in implicit relational memory when they induced temporary amnesia in healthy participants [31]. Participants were asked to perform the contextual cueing task once after an injection of midazolam, an anesthetic that induces temporary amnesia and presumably medial temporal lobe dysfunction, and once after an injection of saline. Under the influence of midazolam, participants showed no contextual cueing effect, whereas general search performance for both old and new displays improved over time, suggesting that skill learning, a non-relational form of implicit learning, was intact. Neither the contextual cueing effect nor the procedural learning was available to conscious awareness, yet only one of these was affected by drug-induced amnesia, suggesting that the contextual cueing impairment was due to an associative learning deficit, rather than to conscious accessibility. Further support comes from neuropsychological studies, in which amnesic patients revealed deficits in contextual cueing [4], as well as in learning of relations among elements of real-world scenes [35]. Our finding that MCI patients who have medial temporal lobe atrophy also revealed contextual cueing deficits in the presence of unimpaired skill learning provides further evidence in support of the distinction based on the type of processing involved, rather than on accessibility to awareness. These data also add to earlier research in indicating that the distinction is based not merely on whether the information requires relational processing, but also on the kinds of relations to be learned and neural mechanisms recruited. That is, the two tasks used in this study differed in the kinds of relations to be learned, such that SRT involved integration of events over time and relied on the fronto-striatal system, while contextual cueing involved integration over space and depended on the medial temporal lobe system. The finding that MCI patients showed preserved sequence learning but were impaired on contextual cueing suggests there might be additional dissociations based on the type of information to be learned in the relational processing.

The present study contributes in several ways to what is known about implicit learning and MCI. The difference in performance on the two implicit learning tasks based on ApoE genotype and MCI status suggests that distinct neural mechanisms might underlie these two learning systems. These findings also help rule out alternative explanations for the results, such as that the deficits in MCI are due to a general skill learning deficit. For both tasks, MCI patients, even though they were slower overall, still showed as much general skill learning as healthy controls, and on the SRT, they revealed equivalent amounts of sequence-specific learning as did the controls. It was only contextual cueing, which relied on the integrity of the medial temporal lobe system, that was impaired in MCI and also influenced by ApoE genotype in healthy controls. Further, although the sample size is small and thus requires replication, on the contextual learning, which we predicted to be influenced by ApoE, there was enough sensitivity to detect significant differences between groups of healthy controls based on ApoE genotype. Finally, it is important that MCI patients showed preserved learning of subtle sequential regularities. This is particularly of interest because it suggests that adapting to subtle repeating sequential regularities in the environment, even when people are often unaware that they are learning, is preserved in MCI. Therefore, rehabilitation and education programs calling upon such implicit learning mechanisms are likely to be effective and could increase the period during which the patients can be relatively independent.

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